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(54) Title: SECRETED POLYPEPTIDES WITH HOMOLOGY TO XENOPUS CEMENT GLAND PROTEINS

(57) Abstract

The present invention relates to zsig10 polynucleotide and novel zsig10 secreted polypeptides encoded thereby. The zsig10 polypeptides are believed to have antimicrobial, mucous–modulating and/or adhesion–modulating activity and may therefore be used in cell culture to evaluate those activities. The present invention also includes antibodies to the zsig10 polypeptides.

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DESCRIPTION

SECRETED POLYPEPTIDES WITH HOMOLOGY TO XENOPUS CEMENT GLAND PROTEINS

BACKGROUND OF THE INVENTION

Proteins expressed in secretory tissues, including proteins found in mucous or like secretions, or in tissues exposed to external agents may be implicated in secretory function or in development or repair of such tissues. Such exposed tissues include the throat, the mouth, the lungs and the like. Other secretory tissues include the prostate, the intestines and the like. Expression of such proteins may serve protective functions for secretory tissue and/or exposed tissue, acting, for example, as an anti-microbial agent or as a mucous-modulating agent, such as a mucous-clearing or a mucous-degrading agent. Inappropriate expression of such proteins involved in secretory function may cause or connote improper mucous composition or secreted amount. Also, inappropriate expression of such proteins involved in secretory organ development or repair may result in inappropriate proliferation or differentiation of secretory tissue. Such proteins or agonists or antagonists thereof are therefore sought to study, detect, prevent and treat secretory tissue disorders and/or exposed tissue maladies. More specifically, moieties which are components of mucous, modulators of mucous secretion or mucous degradation factors are sought.

Also, anti-microbial protective agents may be directly acting or indirectly acting. Such agents, operating via membrane association or pore forming mechanisms of action, directly attach to the offending microbe. Anti-microbial agents can also act via an enzymatic mechanism, breaking down microbial protective

substances or the cell wall/membrane thereof. Anti-microbial agents capable of inhibiting microorganism growth are also sought. An example of a microbial-associated condition with mucous involvement in humans is the diminution of the defensive properties of the gastroduodenal mucosa by *Helicobacter pylori*, potentially resulting in ulcer formation. See, for example, Beligotskii et al., Klin. Khir. 8: 3-6, 1994.

The cement gland is an ectodermal organ in the head of frog embryos anterior to neural tissue. Two proteins, believed to be secreted by the cement gland, have been discovered and designated XLU82110_1 and XLU76752_1. The sequence of XLU82110_1 was published by direct submission without accompanying data.

The amphibian cement gland appears to be involved in anterior/posterior axis formation and may play other roles in amphibian embryogenesis, such as in neural development. In addition, Otte et al., Nature 334: 618-20, 1988, have shown a correlation between neural induction and protein kinase C activation.

In addition, the cement gland is a mucous-secreting organ, which attaches the embryo to a solid support before swimming and feeding begin and provides sensory signals to the embryo to stop moving once such attachment is made. In this manner, the embryo ceases to move, thereby drawing less attention from potential predators. Before feeding begins, the cement gland is undergoes apoptosis. Proteins secreted by the cement gland may also be involved in preparing the substrate for attachment and/or protecting the embryo from microbial attack.

Thus, proteins secreted by the cement gland may have anti-microbial activity and/or be involved in adhesion, differentiation or neural development. Mammalian homologs of such proteins may be useful for anti-microbial applications and/or mucous-modulating

functions. In addition, such homologs or antagonists or agonists thereof are expected to be useful in circumstances where enhancement (homolog or agonist) or inhibition (antagonist) of adhesion is desired. For example, inhibition of microbial pathogen-cell adhesion and pathological tissue adhesions is desired.

In addition, surgical wounds require closure and increased interest has been devoted to the use of biological adhesives to replace or augment the use of mechanical closure devices. Proteins having adhesive properties are sought for such "tissue glue" applications.

The present invention provides such polypeptides for these and other uses that should be apparent to those skilled in the art from the teachings herein.

SUMMARY OF THE INVENTION

Within one aspect, the invention provides an isolated polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 21-175 of SEQ ID NO:2. Within one embodiment the polypeptide is at least 90% identical in amino acid sequence to residues 21-175 of SEQ ID NO:2. Within another embodiment the polypeptide further comprises a cysteine residue corresponding to amino acid residue 81 of SEQ ID NO:2. Within another embodiment the polypeptide further comprises a copper binding site corresponding to amino acid residues 74-78 of SEQ ID NO:2. Within another embodiment the polypeptide comprises residues 26-175 of SEQ ID NO:2. Within another embodiment the polypeptide comprises residues 21-175 of SEQ ID NO:2. Within another embodiment the polypeptide comprises residues 1-175 of SEQ ID NO:2. Within yet another embodiment the polypeptide is at least 1 kb in length. Within another embodiment, the polypeptide is covalently linked to a moiety selected from the group consisting of

affinity tags, toxins, radionucleotides, enzymes and fluorophores. Within a related embodiment the moiety is an affinity tag selected from the group consisting of polyhistidine, FLAG, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region. Within yet another related embodiment there is a proteolytic cleavage site between said sequence of amino acid residues and said affinity tag.

Within another aspect, the invention provides a DNA construct encoding a polypeptide fusion, said fusion comprising a secretory signal sequence having the amino acid sequence of amino acid residues 1-20 of SEQ ID NO:2, wherein said secretory signal sequence is operably linked to an additional polypeptide.

Within another aspect, the invention provides an expression vector comprising the following operably linked elements: a transcription promoter; a DNA segment encoding a polypeptide as described above; and a transcriptional terminator. Within one embodiment the DNA segment further encodes a secretory signal sequence operably linked to said polypeptide. Within a related embodiment the DNA segment encodes the secretory signal sequence having the amino acid sequence of residues 1-20 of SEQ ID NO:2. Within a further related embodiment is provided a cultured cell into which has been introduced the expression vector described above, wherein the cell expresses the polypeptide encoded by the DNA segment.

Within another aspect, the invention provides a method of producing a protein comprising: culturing a cell into which has been introduced an expression vector as described above whereby the cell expresses the protein encoded by the DNA segment; and recovering the expressed protein.

Within other aspects of the invention are provided, a pharmaceutical composition comprising a polypeptide as described above in combination with a

pharmaceutically acceptable vehicle. An antibody that specifically binds to an epitope of a polypeptide as described above. A binding protein that specifically binds to an epitope of a polypeptide as described above.

Within another aspect, the invention provides an isolated polynucleotide encoding a polypeptide as described above. Within one embodiment the polynucleotide is selected from the group consisting of, a) a sequence of nucleotides from nucleotide 138 to nucleotide 587 of SEQ ID NO:1; b) a sequence of nucleotides from nucleotide 123 to nucleotide 587 of SEQ ID NO:2; c) a sequence of nucleotides from nucleotide 63 to nucleotide 587 of SEQ ID NO:2; d) allelic variants of a), b), or c); and e) nucleotide sequences complementary to a), b), c) or d). Within another embodiment the polynucleotide is from 742 to 881 nucleotides in length. Within another embodiment is provided an isolated polynucleotide comprising nucleotide 1 to nucleotide 525 of SEQ ID NO:14. Within another embodiment the polynucleotide is DNA.

Within another aspect is provided an oligonucleotide probe or primer comprising 14 contiguous nucleotides of a polynucleotide of SEQ ID NO:14 or a sequence complementary to SEQ ID NO:14.

Within another aspect, the invention provides method for detecting a genetic abnormality in a patient, comprising: obtaining a genetic sample from a patient; incubating the genetic sample with a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1, under conditions wherein said polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first reaction product; comparing said first reaction product to a control reaction product, wherein a difference between said first reaction product and said control reaction product is indicative of a genetic abnormality in the patient.

Within another aspect, the invention provides a method for detecting zsig10 polypeptides comprising: exposing a polypeptide containing sample to an antibody attached to a solid support, wherein said antibody binds to an epitope of a zsig10 polypeptide; washing said immobilized antibody-polypeptide to remove unbound contaminants; exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig10 polypeptide, wherein the second antibody is associated with a detectable label; and detecting the detectable label.

These and other aspects of the invention will become evident upon reference to the following detailed description of the invention and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 schematically depicts the zsig10 polypeptide structure, with "M" indicating the initial methionine residue; "Signal" indicating a secretory peptide through amino acid residue 20; and the solid bar encompassing amino acid residue 21 to residue 175 indicating a polypeptide homologous to a *Xenopus* secreted protein, with "α" and "β" indicating three alpha helical and five beta sheet structural domains, "~Cu" indicating a putative copper binding site at amino acid residues 74-78 and "C" indicating a free cysteine residue at position 81 (which may constitute part of an intra-chain disulfide in active form or may be an essential moiety for catalytic activity).

Figure 2 illustrates a multiple alignment of two *Xenopus laevis* secreted proteins (XLU821 (SEQ ID NO:3), which corresponds to an abbreviation of XLU82110_1 in published literature and XLU767 (SEQ ID NO:4), which corresponds to XLU76752_1) and a zsig10 polypeptide (SEQ ID NO:2) of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention in detail, it may be helpful to the understanding thereof to define the following terms:

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952-4, 1985), substance P, Flag™ peptide (Hopp et al., Biotechnology 6:1204-10, 1988), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

The term "allelic variant" denotes any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides and proteins. Where the context allows,

these terms are used with reference to a particular sequence or portion of a polypeptide or protein to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a protein is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete protein.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of $<10^9$ M^{-1}.

The term "complements of polynucleotide molecules" denotes polynucleotide molecules having a complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a polynucleotide that has a contiguous stretch of identical or complementary sequence to another polynucleotide. Contiguous sequences are said to "overlap" a given stretch of polynucleotide sequence either in their entirety or along a partial stretch of the polynucleotide. For example, representative contigs to the polynucleotide sequence 5'-ATGGCTTAGCTT-3' are 5'-TAGCTTgagtct-3' and 3'-gtcgacTACCGA-5'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "expression vector" denotes a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments may include promoter and terminator sequences, and may optionally include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide molecule, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316:774-78, 1985). When applied to a protein, the term "isolated" indicates that the protein is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated protein is substantially free

of other proteins, particularly other proteins of animal origin. It is preferred to provide the protein in a highly purified form, i.e., greater than 95% pure, more preferably greater than 99% pure.

The term "operably linked", when referring to DNA segments, denotes that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

The term "polynucleotide" denotes a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired. Such unpaired ends will in general not exceed 20 nt in length.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced

naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

The term "promoter" denotes a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule (i.e., a ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. Most nuclear receptors also exhibit a multi-domain structure, including an amino-terminal,

transactivating domain, a DNA binding domain and a ligand binding domain. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger peptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (e.g., gel electrophoresis) will be understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a polypeptide having homology to two secreted proteins found in *Xenopus laevis* (SEQ ID NO: 3; XLU82110_1, with Met is at position 1 and SEQ ID NO: 4; XLU76752_1, with Met also

at position 1). The protein of the present invention appears to be a soluble protein formed of alpha helical and beta sheet structures (designated "α" and "β" in Fig. 1). Thus, the zsig10 polypeptide is therefore characterized by a mixed alpha helix-beta sheet structure.

In addition, the zsig10 polypeptide of the present invention has a free cysteine residue at position 81 of SEQ ID NO: 2 (designated "C" in Fig. 1) and a putative copper binding site at amino acids 74-78 of SEQ ID NO: 2 (designated "~Cu" in Fig. 1). This copper binding motif most closely matches the cytochrome C oxidase subunit I copper B binding site. The presence of a free cysteine may indicate that the zsig10 polypeptide forms homodimers or heterodimers via disulfide bond formation. More specifically, the cysteine residue may constitute part of an intra-chain disulfide in active form. Zsig10 polypeptide homodimers and zsig10 polypeptide-containing proteinaceous heterodimers are also contemplated by the present invention. Alternatively, the free cysteine may be an essential moiety necessary for catalytic activity.

The zsig10 polypeptides of the present invention also preferably incorporate six potential protein kinase C phosphorylation sites, at amino acids 24, 68, 114, 136, 142 and 146 of SEQ ID NO: 2. Such putative sites of phosphorylation may indicate that the zsig10 polypeptides of the present invention are involved in neural induction, since a correlation between protein kinase C activity and induction has been noted. See Otte et al. referenced above. In addition, the zsig10 polypeptides of the present invention preferably incorporate one potential casein kinase II phosphorylation site at amino acid 57 of SEQ ID NO: 2. Such a phosphorylation site may impact zsig10 polypeptide *in vivo* half-life or localization, protein-protein interaction or function.

Analysis of the tissue distribution of the mRNA corresponding to this novel DNA by both Northern blot and Dot blot showed that expression was highest in lung, prostate, small intestine, colon, trachea and stomach, followed by apparent but decreased expression levels in uterus, pancreas and kidney. Two transcript sizes were observed, one at approximately 1 kb and one at approximately 2 kb. The 1 kb message was detected in much higher abundance than the 2 kb message, with the 1 kb message expressed at least about 50 times higher in most tissues except trachea where the expression appeared to be approximately 25 times higher. The polynucleotide sequence in SEQ ID NO: 1 appears to correspond to the 1 kb message. The polypeptide encoded by that polynucleotide sequence has been designated zsig10.

The novel zsig10 polypeptide-encoding polynucleotides of the present invention were initially identified by querying an EST database for secretory signal sequences characterized by an upstream methionine start site, a hydrophobic region of approximately 13 amino acids and a cleavage site (SEQ ID NO: 5, wherein cleavage occurs between the alanine and arginine amino acid residues) in an effort to select for secreted proteins. Polypeptides corresponding to ESTs meeting those search criteria were compared to known sequences to identify secreted proteins having homology to known ligands. A single EST sequence was discovered and predicted to be a secreted protein. Full length sequencing thereof allowed discovery of a homolog relationship to two secreted proteins found in *Xenopus laevis* (XLU82110_1 and XLU76752_1). See, for example, Sive et al., Dev. Dyn. 205(3): 265-80, 1996 and Sive et al., Cell 58(1): 171-80, 1989.

The full sequence of the zsig10 polypeptide was obtained from a single clone believed to contain it, wherein the clone was obtained from a small intestine

tissue library. Other libraries that might also be searched for such clones include colon, ovary, prostate, stomach, fetal liver and/or spleen, small intestine, trachea, lung, fetal lung and the like.

The full length nucleotide sequence encoding zsig10 polypeptide is described in SEQ ID NO:1, and its deduced amino acid sequence is described in SEQ ID NO:2. When aligned as in Fig. 2, aligned positions 83-87 (amino acids 74-78 of the polypeptide of SEQ ID NO:2) showed one conservative amino acid substitution (valine for isoleucine) at position 84 (74 in SEQ ID NO:2). Thus, the aligned polypeptides appear to share a putative copper binding site. Also, Fig. 2 shows that the aligned proteins share a free cysteine at aligned position 90 (position 81 of SEQ ID NO:2). In addition, XLU82110_1 (SEQ ID NO:3) and XLU76752_1 (SEQ ID NO:4) appear to share the mixed alpha helix/beta sheet structure characteristic of zsig10 polypeptides.

Analysis of the DNA encoding a zsig10 polypeptide (SEQ ID NO:1) revealed an open reading frame encoding 175 amino acids (SEQ ID NO:2) comprising a signal peptide of 20 amino acid residues (residue 1 to residue 20 of SEQ ID NO:2) and a mature polypeptide of 155 amino acids (residue 21 to residue 175 of SEQ ID NO:2). Those skilled in the art will recognize that predicted secretory signal sequence domain boundaries are approximations based on primary sequence content, and may vary slightly; however, such estimates are generally accurate to within ± 4 amino acid residues. Therefore the present invention also includes the polypeptides having amino acid sequences comprising amino acid residues 17-175 of SEQ ID NO:2, residues 18-175 of SEQ ID NO:2, residues 19-175 of SEQ ID NO:2, residues 20-175 of SEQ ID NO:2, residues 21-175 of SEQ ID NO:2, residues 22-175, residues 23-175 of SEQ ID NO:2 and residues 24-175 of SEQ ID NO:2 as well as the polynucleotides encoding them. The C-terminal tail of the

zsig10 polypeptide appears to be longer than that of XLU82110_1 (SEQ ID NO:3) and about the same length as that of XLU76752_1 (SEQ ID NO:4). Also, the C-terminal tail region of aligned proteins zsig10 (SEQ ID NO:2) and XLU76752_1 (SEQ ID NO:4), but not XLU82110_1 (SEQ ID NO:3), is highly positively charged, potentially indicating alternative regulation or specificity.

Multiple alignment of zsig10 polypeptide (SEQ ID NO:2) with *Xenopus laevis* secreted proteins XLU821 (SEQ ID NO:3) (abbreviation for XLU82110_1) and XLU767 (SEQ ID NO:4) (abbreviation for XLU76752_1), as shown in Fig. 2, revealed a block of high percent identity ranging from aligned amino acid residue 46 to residue 173. Within the region of high identity, the following percent identity figures are observed for the deduced amino acid sequence of SEQ ID NO:2, XLU82110_1 (SEQ ID NO:3) and XLU76752_1 (SEQ ID NO:4).

	Zsig10	*Xenopus* XLU76752_1	*Xenopus* XLU82110_1
Zsig10	100	52	56
Xenopus XLU76752_1	52	100	95
Xenopus XLU82110_1	56	95	100

The highly conserved amino acids, both within and without the region of high identity, can be used as a tool to identify zsig10 polypeptides or zsig10-like proteins. For instance, reverse transcription-polymerase chain reaction (RT-PCR) can be used to amplify sequences encoding the conserved motifs suggested by the multiple alignment from RNA obtained from a variety of tissue sources. In particular, the following primers are useful for this purpose:

1) Amino acids 20-25 of SEQ ID NO: 2 (corresponding to nucleotides 63-137 of SEQ ID NO:1, nucleotides 58-75 of SEQ ID NO:14 and their complements);

2) Amino acids 37-42 of SEQ ID NO: 2 (corresponding to nucleotides 387-437 of SEQ ID NO:1, nucleotides 109-126 of SEQ ID NO:14 and their complements);

3) Amino acids 85-90 of SEQ ID NO: 2 (corresponding to nucleotides 315-332 of SEQ ID NO:1, nucleotides 253-270 of SEQ ID NO:14 and their complements);

4) Amino acids 50-55 of SEQ ID NO: 2 (corresponding to nucleotides 210-227 of SEQ ID NO:1, nucleotides 148-165 of SEQ ID NO:14 and their complements);

5) Amino acids 115-120 of SEQ ID NO: 2 (corresponding to nucleotides 405-422 of SEQ ID NO:1, nucleotides 343-260 of SEQ ID NO:14 and their complements); and

6) Amino acids 43-48 of SEQ ID NO: 2 (corresponding to nucleotides 189-206 of SEQ ID NO:1, nucleotides 127-144 of SEQ ID NO:14 and their complements).

The activity of polypeptides identified by such probes or of polypeptides encoded by polynucleotides identified by such probes can be determined by methods that are known in the art as generally described herein.

Oligonucleotide probes based on the polynucleotide sequence of SEQ ID NO:1 can be used to localize the zsig10 gene to a particular chromosome. Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes (Cox et al., Science 250:245-50, 1990). Partial or full knowledge of a gene's sequence allows one to design PCR primers suitable

for use with chromosomal radiation hybrid mapping panels. Radiation hybrid mapping panels are commercially available which cover the entire human genome, such as the Stanford G3 RH Panel and the GeneBridge 4 RH Panel (Research Genetics, Inc., Huntsville, AL). These panels enable rapid, PCR-based chromosomal localizations and ordering of genes, sequence-tagged sites (STSs), and other nonpolymorphic and polymorphic markers within a region of interest. This includes establishing directly proportional physical distances between newly discovered genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

Sequence tagged sites (STSs) can also be used independently for chromosomal localization. An STS is a DNA sequence that is unique in the human genome and can be used as a reference point for a particular chromosome or region of a chromosome. An STS is defined by a pair of oligonucleotide primers that are used in a polymerase chain reaction to specifically detect this site in the presence of all other genomic sequences. Since STSs are based solely on DNA sequence they can be completely described within an electronic database, for example, Database of Sequence Tagged Sites (dbSTS), GenBank, (National Center for Biological Information, National Institutes of Health, Bethesda, MD http://www.ncbi.nlm.nih.gov), and can be searched with a gene sequence of

interest for the mapping data contained within these short genomic landmark STS sequences.

The results of chromosome mapping experiments, as more fully described in Example 3 hereof, showed that the zsig10 gene maps 59.99 cR from the top of the human chromosome 7 linkage group on the WICGR radiation hybrid map. Relative to the centromere, its nearest proximal marker was AFM144ZA1 and its nearest distal maker was WI-11644. The use of surrounding markers positioned the zsig10 gene in the 7p21.1-p15.3 region on the integrated LDB chromosome 7 map.

The present invention also provides polynucleotide molecules, including DNA and RNA molecules, that encode the zsig10 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:14 is a degenerate DNA sequence that encompasses all DNAs that encode the zsig10 polypeptide of SEQ ID NO:2. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:14 also provides all RNA sequences encoding SEQ ID NO:2 by substituting U for T. Thus, zsig10 polypeptide-encoding polynucleotides comprising nucleotide 1 to nucleotide 525 of SEQ ID NO:14 and their RNA equivalents are contemplated by the present invention. Table 1 sets forth the one-letter codes used within SEQ ID NO:14 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

TABLE 1

Nucleotide	Resolution	Nucleotide	Complement
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

The degenerate codons used in SEQ ID NO:14, encompassing all possible codons for a given amino acid, are set forth in Table 2.

TABLE 2

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described herein.

One of ordinary skill in the art will also appreciate that different species can exhibit "preferential codon usage." In general, see, Grantham, et al., Nuc. Acids Res. 8:1893-912, 1980; Haas, et al. Curr. Biol. 6:315-24, 1996; Wain-Hobson, et al., Gene 13:355-64, 1981; Grosjean and Fiers, Gene 18:199-209, 1982; Holm, Nuc. Acids Res. 14:3075-87, 1986; Ikemura, J. Mol. Biol. 158:573-97, 1982. As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 2). For example, the amino acid Threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential

codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequence disclosed in SEQ ID NO:14 serves as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

Based upon the homology of zsig10 polypeptides to proteins secreted by amphibian cement gland, zsig10 polypeptides may be involved in differentiation of neural tissue, such as in anterior/posterior axis formation. As stated above, zsig10 polypeptides of the present invention bear six putative protein kinase C phosphorylation sites. Otte et al., Nature 334:618-20, 1988, have shown a correlation between neural induction and protein kinase C activation. Consequently, zsig10 polypeptides may be useful to evaluate the potential of mammalian neural tissue to grow, develop or differentiate.

In addition, the cement gland is a mucous-secreting organ. Thus, zsig10 polypeptides may be involved in adhesion or other mucous-mediated functions. More specifically, zsig10 polypeptides may constitute a component of mucous or may be a factor influencing mucous production, mucous composition or mucous integrity. Zsig10 polypeptides may also serve a mucous-clearing function in conditions associated with pathological mucous deposition.

Consequently, zsig10 polypeptides or antagonists or agonists thereof are expected be useful in circumstances where modulation of adhesion is desired. Such adhesion-modulating function may be used in *in vitro* experiments designed to study adhesion, such as inhibition of adhesion of microorganisms to cells, tissue or mucous.

Enhancers and inhibitors of adhesion also have potential as therapeutics for conditions requiring such enhancement or inhibition. For example, enhanced tumor cell-tumor cell adhesion in a primary solid tumor does not favor metastasis thereof. Also, diminished tumor cell-endothelial cell adhesion also does not favor metastasis formation at a site distant from the primary tumor. Assays to assess metastatic potential, as exemplified by adhesion, are known in the art. See, for example, Koenigsmann et al., Onkologie 17: 528-37, 1994, Asao et al., Cancer Letters 78: 57-62, 1994 and the like. Adhesion may also be evaluated in assays assessing mucous samples for known indicia of adhesion, such as bacterial colonization, susceptibility to and persistence of infection and the like.

In addition, zsig10 polypeptides or agonists or antagonists thereof are expected to be useful in the modulation of mucous production, composition or integrity or in a mucous clearing role. Such modulation may be useful in altering mucous composition or integrity for *in vitro* study thereof, such as reducing integrity of mucous to evaluate the implication thereof on bacterial-mucous interaction. In addition, such modulation may be useful in the treatment of disease states characterized by inappropriate mucous production, composition or integrity. For example, cystic fibrosis is associated with dehydration of the mucous, which results in mucous thickening (reduction in viscosity). Other conditions, such as chronic obstructive pulmonary disease, asthma, and the like, are associated with chronic mucous hypersecretion. See, for example, Prescott et al., Ugeskr Laeger 158(45): 6456-60, 1996; Gordon, Ear Nose Throat J. 75(2): 97-101, 1996; and Jeffery, Am. J. Respir. Crit. Care Med. 150(5 Pt 2): S6-13, 1994. Also, chronic obstructive pulmonary disease and sinonasal inflammatory disease are associated with changes in rhealogical

properties or thickening of mucous. See, for example, Agliati, J. Int. Med. Res. 24(3): 302-10, 1996 and Wippold et al., Allergy Proc. 16(4): 165-9, 1995. In addition, mucous structural integrity is adversely impacted in inflammatory bowel disease, possibly via increased proteolysis. See, for example, Playford et al., Amer. J. Pathol. 146(2): 310-6, 1995. Certain forms of chronic obstructive pulmonary disease are associated with increased acidic mucous. See, for example, the Jeffery article cited above. Mucous clearing may be useful in a number of these conditions as well.

To verify these capabilities in zsig10 polypeptides, agonists or antagonists of the present invention, such zsig10 polypeptides, agonists or antagonists are evaluated for mucosal integrity maintenance activity according to procedures known in the art. See, for example, Zahm et al., Eur. Respir. J. 8: 381-6, 1995, which describes methods for measuring viscoelastic properties and surface properties of mucous as well as for evaluating mucous transport by cough and by ciliary activity. If desired, zsig10 polypeptide performance in this regard can be compared to mucins or the like. Other assays for evaluating the properties of mucous are known to those of ordinary skill in the art. Such assays include those for determining mucin content, water content, carbohydrate content, intrinsic buffering capacity, acidity, barrier properties, ability to absorb water and the like.

Moreover, detection of zsig10 polypeptides in the serum, mucous or tissue biopsy of a patient undergoing evaluation for or disorders characterized by inappropriate mucous deposition, composition or properties, such as cystic fibrosis, asthma, bronchitis, inflammatory bowel disease, Crohn's disease, chronic obstructive pulmonary disease or the like, can be employed in a diagnostic application of the present invention. Such zsig10 polypeptides can be detected using immunoassay techniques

and antibodies capable of recognizing a zsig10 polypeptide epitope. More specifically, the present invention contemplates methods for detecting zsig10 polypeptide comprising:

exposing a sample possibly containing zsig10 polypeptide to an antibody attached to a solid support, wherein said antibody binds to an epitope of a zsig10 polypeptide;

washing said immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig10 polypeptide, wherein the second antibody is associated with a detectable label; and

detecting the detectable label. Elevated concentrations of zsig10 polypeptide (in comparison to normal concentrations thereof) in the test sample appears to be indicative of dysfunction.

In addition, pharmaceutical compositions containing such mucosa-modulating agents may be employed in the treatment of disorders associated with alterations in mucosal production, composition or integrity, such as those described above. Such patients will be given an effective amount of zsig10 polypeptide or agonist or antagonist thereof having mucosal-modulating activity to achieve a therapeutic benefit, generally manifested in a change in mucosal production, composition or integrity in the direction of the normal physiological state thereof.

Also, the zsig10 polypeptides of the present invention are found in high abundance in digestive tissues, such as stomach, small intestine and colon. Thus, expression of zsig10 polypeptides may serve as a marker for digestive function or to promote digestive organ proliferation or differentiation. Also, zsig10 polypeptides or agonists or antagonists thereof may be useful in modulating the lubrication or barrier properties of digestive organ mucosa.

Zsig10 polypeptides of the present invention or agonists or antagonists thereof may be used as anti-microbial agents to protect against pathological action of microorganisms. Such anti-bacterial agents are preferably active on mucosa-associated microorganisms, such as *C. albicans*, *pneumonus*, *hemophilus*, *H. pylori*, and the like. An example of a microbial-associated condition with mucous involvement in humans is the diminution of the defensive properties of the gastroduodenal mucosa by *Helicobacter pylori*, potentially resulting in ulcer formation. See, for example, Beligotskii et al., Klin. Khir. 8: 3-6, 1994.

These anti-microbial protective agents may be directly acting or indirectly acting. Such agents operating via membrane association or pore forming mechanisms of action directly attach to the offending microbe. Anti-microbial agents can also act via an enzymatic mechanism, breaking down microbial protective substances or the cell wall/membrane thereof. Anti-microbial agents, capable of inhibiting microorganism proliferation or action or of disrupting microorganism integrity by either mechanism set forth above, are useful in methods for preventing contamination in cell culture by microbes susceptible to that anti-microbial activity. Such techniques involve culturing cells in the presence of an effective amount of said zsig10 polypeptide or an agonist or antagonist thereof. Assays to determine the capability of zsig10 polypeptides or agonist or antagonists thereof as anti-microbial agents are known in the art.

Moreover, detection of zsig10 polypeptides in the serum, mucous or tissue biopsy of a patient undergoing evaluation for microbial disorders, particularly those associated with mucosa, can be employed in a diagnostic application of the present invention. Such zsig10 polypeptides can be detected using immunoassay techniques and antibodies capable of recognizing a zsig10 polypeptide

epitope. More specifically, the present invention contemplates methods for detecting zsig10 polypeptide comprising:

exposing a sample possibly containing zsig10 polypeptide to an antibody attached to a solid support, wherein said antibody binds to an epitope of a zsig10 polypeptide;

washing said immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig10 polypeptide, wherein the second antibody is associated with a detectable label; and

detecting the detectable label. Depressed concentrations of zsig10 polypeptide (in comparison to normal concentrations thereof) in the test sample appears to be indicative of dysfunction.

In addition, pharmaceutical compositions containing such anti-microbial agents may be employed in the treatment of microbial disorders, particularly those associated with mucosa. Such patients will be given an effective amount of zsig10 polypeptide or agonist or antagonist thereof having anti-microbial activity to achieve a therapeutic benefit, generally manifested in a decrease in proliferation or function of the pathogenic microbe. Other conditions which may be addressed in accordance with the present invention are eye, nasal, oral and rectal conditions involving the mucosa and/or pathological microbial agents, chemotherapy side effects impacting the mucosa, AIDS complications relating to mucosa or the like. The anti-microbial activity of zsig10 polypeptides, agonists or antagonists may be determined using known assays therefore. See, for example, Barsum et al., Eur. Respir. J. 8(5): 709-14, 1995; Sandovsky-Losica et al., J. Med. Vet. Mycol (England) 28(4): 279-87, 1990; Mehentee et al., J. Gen. Microbiol (England) 135 (Pt. 8):

2181-8, 1989; Segal and Savage, Journal of Medical and Veterinary Mycology 24: 477-479, 1986 and the like.

Also, zsig10 polypeptides of the present invention may also constitute a component of a known tissue glue, imparting additional adhesive and/or anti-microbial properties thereto. In such applications, purified zsig10 polypeptide would be used in combination with collagen or a form of gelatin, muscle adhesion protein, fibrinogen, thrombin, Factor XIII or the like. The different types of tissue glues as well as the composition thereof are known in the art.

The present invention provides methods for identifying agonists or antagonists of the zsig10 polypeptides disclosed above, which agonists or antagonists may have valuable therapeutic properties as discussed further herein. Within one embodiment, there is provided a method of identifying zsig10 polypeptide agonists, comprising providing cells responsive to a zsig10 polypeptide as disclosed above, culturing the cells in the presence of a test compound and comparing the cellular response with the cell cultured in the presence of the zsig10 polypeptide, and selecting the test compounds for which the cellular response is of the same type. Agonists are therefore useful to mimic or augment the function of zsig10 polypeptides.

Within another embodiment, there is provided a method of identifying antagonists of zsig10 polypeptide, comprising providing cells responsive to a zsig10 polypeptide, culturing a first portion of the cells in the presence of zsig10 polypeptide, culturing a second portion of the cells in the presence of the zsig10 polypeptide and a test compound, and detecting a decrease in a cellular response of the second portion of the cells as compared to the first portion of the cells. Antagonists are therefore useful to inhibit or diminish zsig10 polypeptide function.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar

sized regions of SEQ ID NO:1, SEQ ID NO: 6 (an oligonucleotide primer designated ZC11668), SEQ ID NO: 7 (an oligonucleotide primer designated ZC12253), SEQ ID NO: 8 (an oligonucleotide primer designated ZC12241), other probes described herein, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is less than about 0.02 M at pH 7 and the temperature is at least about 60°C.

As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from fetal liver or spleen, colon, ovary, prostate, stomach, small intestine, lung, fetal lung or trachea, although DNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly $(A)^+$ RNA is prepared from total RNA using the method of Aviv and Leder (Proc. Natl. Acad. Sci. USA 69:1408-12, 1972). Complementary DNA (cDNA) is prepared from poly$(A)^+$ RNA using known methods. Polynucleotides encoding zsig10 polypeptides are then identified and isolated by, for example, hybridization or PCR.

The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of

particular interest are zsig10 polypeptides from other mammalian species, including murine, rat, porcine, ovine, bovine, canine, feline, equine and other primate proteins. Orthologs of the human proteins can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the protein. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue of cell line. A zsig10-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent 4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to zsig10 polypeptide. Similar techniques can also be applied to the isolation of genomic clones.

Those skilled in the art will recognize that the sequences disclosed in SEQ ID NO:1 and SEQ ID NO:2 represent a single allele of the human zsig10 gene and polypeptide, and that allelic variation and alternative splicing are expected to occur. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the zsig10 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. For example, Northern blot analysis revealed 1 kb and 2 kb mRNAs, wherein the 1 kb variant was more highly expressed (approximately 50 times higher in most tissues and approximately 25 times higher in

trachea). Such mRNA species are likely to be splice variants. In addition, allelic variants and splice variants can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO: 1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention.

The present invention also provides isolated zsig10 polypeptides that are substantially homologous to the polypeptides of SEQ ID NO:2 and their species homologs/orthologs. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2 or their orthologs. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2 or its orthologs. Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-9, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

Table 3

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 4) and other substitutions that do not significantly affect the folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), or the like. Preferred such sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 4

Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

The present invention further provides a variety of other polypeptide fusions [and related multimeric proteins comprising one or more polypeptide fusions]. For example, a zsig10 polypeptide can be prepared as a fusion to a dimerizing protein as disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Preferred dimerizing proteins in this regard include immunoglobulin constant region domains. Immunoglobulin-zsig10 polypeptide fusions can be expressed in genetically engineered cells [to produce a variety of multimeric zsig10 analogs]. Auxiliary domains can be fused to zsig10 polypeptides to target them to specific cells, tissues, or macromolecules (e.g., collagen). For example, a zsig10 polypeptide or protein could be targeted to a predetermined cell type by fusing a zsig10 polypeptide to a ligand that specifically binds to

a receptor on the surface of the target cell. In this way, polypeptides and proteins can be targeted for therapeutic or diagnostic purposes. A zsig10 polypeptide can be fused to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., Con. Tiss. Res. 34:1-9, 1996.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically

aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for zsig10 amino acid residues.

Essential amino acids in the zsig10 polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-5, 1989). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (e.g., adhesion modulation, anti-microbial activity or the like) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., J. Biol. Chem. 271:4699-708, 1996. Sites of ligand-receptor interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of

putative contact site amino acids. See, for example, de Vos et al., Science 255:306-312, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related polypeptides.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-57, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-2156, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-7, 1991; Ladner et al., U.S. Patent NO: 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Variants of the disclosed zsig10 DNA and polypeptide sequences can be generated through DNA shuffling as disclosed by Stemmer, Nature 370:389-91, 1994, Stemmer, Proc. Natl. Acad. Sci. USA 91:10747-51, 1994 and WIPO Publication WO 97/20078. Briefly, variant DNAs are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNAs, such as allelic variants or DNAs from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of

sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed above can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides (e.g., capable of modulating adhesion, having anti-microbial activity or the like) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed above, one of ordinary skill in the art can identify and/or prepare a variety of polypeptides that are substantially homologous to residues 21-175 of SEQ ID NO:2 or allelic variants thereof and retain the adhesion-modulating, anti-microbial or like properties of the wild-type protein. Such polypeptides may include additional amino acids, such as affinity tags and the like. Such polypeptides may also include additional polypeptide segments as generally disclosed herein.

The polypeptides of the present invention, including full-length proteins, fragments thereof and fusion proteins, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory

Press, Cold Spring Harbor, NY, 1989, and Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987.

In general, a DNA sequence encoding a zsig10 polypeptide of the present invention is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a zsig10 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the zsig10 polypeptide, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is joined to the zsig10 DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent NO: 5,037,743; Holland et al., U.S. Patent NO: 5,143,830). Conversely, the secretory signal sequence portion of the zsig10 polypeptide (amino acids 1-20 of SEQ

ID NO: 2) may be employed to direct the secretion of an alternative protein by analogous methods.

Cultured mammalian cells are also preferred hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981: Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann et al., EMBO J. 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987), liposome-mediated transfection (Hawley-Nelson et al., Focus 15:73, 1993; Ciccarone et al., Focus 15:80, 1993), and viral vectors (Miller and Rosman, BioTechniques 7:980-90, 1989; Wang and Finer, Nature Med. 2:714-6, 1996). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent NO: 4,713,339; Hagen et al., U.S. Patent NO: 4,784,950; Palmiter et al., U.S. Patent NO: 4,579,821; and Ringold, U.S. Patent NO: 4,656,134. Preferred cultured mammalian cells include the COS-1 (ATCC NO: CRL 1650), COS-7 (ATCC NO: CRL 1651), BHK 570 (ATCC NO: CRL 10314), 293 (ATCC NO: CRL 1573; Graham et al., J. Gen. Virol. 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC NO: CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent NO: 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been

inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g., hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent NO: 5,162,222; Bang et al., U.S. Patent NO: 4,775,624; and WIPO publication WO 94/06463.

Insect cells can be infected with recombinant baculovirus, commonly derived from Autographa californica

nuclear polyhedrosis virus (AcNPV). DNA encoding the zsig10 polypeptide is inserted into the baculoviral genome in place of the AcNPV polyhedrin gene coding sequence by one of two methods. The first is the traditional method of homologous DNA recombination between wild-type AcNPV and a transfer vector containing the zsig10 flanked by AcNPV sequences. Suitable insect cells, e.g. SF9 cells, are infected with wild-type AcNPV and transfected with a transfer vector comprising a zsig10 polynucleotide operably linked to an AcNPV polyhedrin gene promoter, terminator, and flanking sequences. See, King and Possee, The Baculovirus Expression System: A Laboratory Guide, London, Chapman & Hall; O'Reilly et al., Baculovirus Expression Vectors: A Laboratory Manual, New York, Oxford University Press., 1994; and Richardson, C. D., Ed., Baculovirus Expression Protocols. Methods in Molecular Biology, Totowa, NJ, Humana Press, 1995. Natural recombination within an insect cell will result in a recombinant baculovirus which contains zsig10 driven by the polyhedrin promoter. Recombinant viral stocks are made by methods commonly used in the art.

The second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow et al., J. Virol. 67:4566-79, 1993). This system is sold in the Bac-to-Bac kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the zsig10 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case zsig10. However, pFastBac1™ can be modified to a considerable degree. The polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as *Pcor*, p6.9 or MP promoter) which

is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins. See, Hill-Perkins and Possee, J. Gen. Virol. 71:971-6, 1990; Bonning et al., J. Gen. Virol. 75:1551-6, 1994; and, Chazenbalk, G.D., and Rapoport, B., J. Biol. Chem. 270:1543-9, 1995. In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native zsig10 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus gp67 (PharMingen, San Diego, CA) can be used in constructs to replace the native zsig10 secretory signal sequence. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed zsig10 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952-4, 1985). Using a technique known in the art, a transfer vector containing zsig10 is transformed into *E. coli*, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, e.g. Sf9 cells. Recombinant virus that expresses zsig10 is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *S. frugiperda*. See, in general, Glick and Pasternak, Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press, Washington, D.C., 1994. Another suitable cell line is the High FiveO™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S.

Patent #5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cell0405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. The cells are grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. The recombinant virus-infected cells typically produce the recombinant zsig10 polypeptide at 12-72 hours post-infection and secrete it with varying efficiency into the medium. The culture is usually harvested 48 hours post-infection. Centrifugation is used to separate the cells from the medium (supernatant). The supernatant containing the zsig10 polypeptide is filtered through micropore filters, usually 0.45 μm pore size. Procedures used are generally described in available laboratory manuals (King and Possee, ibid.; O'Reilly et al., ibid.; Richardson, ibid.). Subsequent purification of the zsig10 polypeptide from the supernatant can be achieved using methods described herein.

Fungal cells, including yeast cells, and particularly cells of the genus *Saccharomyces*, can also be used within the present invention, such as for producing zsig10 fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent NO: 4,599,311; Kawasaki et al., U.S. Patent NO: 4,931,373; Brake, U.S. Patent NO: 4,870,008; Welch et al., U.S. Patent NO: 5,037,743; and Murray et al., U.S. Patent NO: 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g.,

leucine). A preferred vector system for use in yeast is the POT1 vector system disclosed by Kawasaki et al. (U.S. Patent NO: 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent NO: 4,599,311; Kingsman et al., U.S. Patent NO: 4,615,974; and Bitter, U.S. Patent NO: 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, P. methanolica, P. guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-65, 1986 and Cregg, U.S. Patent NO: 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent NO: 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent NO: 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent NO: 4,486,533.

The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publications WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, it is preferred that the promoter and terminator in the plasmid be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To

facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (*PEP4* and *PRB1*) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (τ) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (see, e.g., Sambrook et al., ibid.). When expressing a zsig10 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of

reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

Expressed recombinant zsig10 polypeptides (or chimeric zsig10 polypeptides) can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred, with DEAE Fast-Flow Sepharose (Pharmacia, Piscataway, NJ) being particularly preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl,

or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, <u>Affinity Chromatography: Principles & Methods</u>, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

The polypeptides of the present invention can be isolated by exploitation of their structural properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins or proteins containing a His tag. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, <u>Trends in Biochem.</u> <u>3</u>:1-7, 1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods

of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (Methods in Enzymol., Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), Acad. Press, San Diego, 1990, pp.529-39). Preferably, a fusion of the polypeptide of interest and an affinity tag (e.g., FLAG, Glu-Glu, polyhistidine, maltose-binding protein, an immunoglobulin domain) or a member of a complement/anti-complement pair may be constructed to facilitate purification. Zsig10 fused to an N- or C-terminal FLAG tag or Glu-Glu tag can be purified by virtue of the affinity tags discussed in more detail in the examples below. Such purification methods allow for purification of proteins where the structural properties are not known or are not amenable to exploitation for purification.

Protein refolding (and optionally reoxidation) procedures may be advantageously used. It is preferred to purify the protein to >80% purity, more preferably to >90% purity, even more preferably >95%, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified protein is substantially free of other proteins, particularly other proteins of animal origin.

Zsig10 polypeptides or fragments thereof may also be prepared through chemical synthesis. Zsig10 polypeptides may be monomers or multimers; glycosylated or non-glycosylated; pegylated or non-pegylated; and may or may not include an initial methionine amino acid residue.

A zsig10-binding polypeptide can also be used for purification of the zsig10 polypeptide of the present invention. The zsig10-binding polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked

agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing zsig10 polypeptide are passed through the column one or more times to allow zsig10 polypeptide to bind to the receptor polypeptide. The bound zsig10 polypeptide is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-40, 1991 and in Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates,

from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Zsig10 polypeptide and other ligand homologs can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, Ann. NY Acad. Sci. 51: 660-72, 1949) and calorimetric assays (Cunningham et al., Science 253:545-48, 1991; Cunningham et al., Science 245:821-25, 1991). In this context, for example, zsig10 polypeptides may modulate the binding of factors or itself constitute a factor involved in the assembly of extracellular matrix or mucous-type secretions.

Zsig10 polypeptides can also be used to prepare antibodies that specifically bind to zsig10 epitopes, peptides or polypeptides. Antibodies generated from this immune response can be isolated and purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, for example, Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995; Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL, 1982.

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, hamsters, guinea pigs and rats as well as transgenic animals such as transgenic sheep, cows, goats or pigs. Antibodies may also be expressed in yeast and fungi in modified forms as well as in mammalian and insect cells. The zsig10 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal or elicit an immune response. Suitable antigens would include the

zsig10 polypeptide encoded by SEQ ID NO:2 from amino acid residue 21-175 of SEQ ID NO:2, or a contiguous 9-175 amino acid residue fragment thereof. The immunogenicity of a zsig10 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of zsig10 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced. Human antibodies can also be made in mice

having a humanized humoral immune system (Mendez et al., Nat. Genet. 14:146- 56, 1997).

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to zsig10 protein or peptide, and selection of antibody display libraries, in phage or similar vectors (for instance, through use of immobilized or labeled zsig10 protein or peptide). Mutagenesis methods discussed herein, in particular domain shuffling, can be used to generate and mature antibodies.

The antibodies of the current invention, or fragments thereof, can be used to direct molecules to a specific target. For example, as T-bodies, chimeric receptors combining antibody recognition with T cell effector function, (Eshhar et al., Springer Semin Immunopathol. 18:199-209, 1996; Eshhar, Cancer Immunol. Immunother. 45:131-6, 1997). Intrabodies, engineered single-chain antibodies expressed inside the cell and having high affinity and specificity for intracellular targets. Such molecules have use in gene therapy and treatment of infectious diseases (Marasco, Immunotechnology 1:1-19, 1995; Marasco et al., Gene Ther. 4:11-5, 1997; Rondon and Marasco, Annu. Rev. Microbiol. 51:257-83, 1997 and Mhashilkar et al., J. Virol. 71:6486-94, 1997). Diabodies, bispecific non-covalent dimers of scFv antibodies useful for immunodiagnosis and therapeutically. In addition they can be constructed in bacteria (Holliger et al., Proc. Natl. Acad. Sci. USA 90:6444-48, 1993).

Antibodies herein specifically bind if they bind to a zsig10 polypeptide, peptide or epitope with a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary

skill in the art, for example, by Scatchard analysis (Scatchard, G., Ann. NY Acad. Sci. 51: 660-72, 1949).

Genes encoding polypeptides having potential zsig10 polypeptide binding domains, "binding proteins", can be obtained by screening random or directed peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. Alternatively, constrained phage display libraries can also be produced. These peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such peptide display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Peptide display libraries can be screened using the zsig10 sequences disclosed herein to identify proteins which bind to zsig10. These "binding proteins" which interact with zsig10 polypeptides can be used essentially like an antibody, for tagging cells; for isolating homolog polypeptides by affinity purification; directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for diagnostic assays for

determining circulating levels of polypeptides; for detecting or quantitating soluble polypeptides as marker of underlying pathology or disease. To increase the half-life of these binding proteins, they can be conjugated. Their biological properties may be modified by dimerizing or multimerizing for use as agonists or antagonists.

A variety of assays known to those skilled in the art can be utilized to detect antibodies and/or binding proteins which specifically bind to zsig10 proteins or peptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant zsig10 protein or polypeptide.

Antibodies and binding proteins to zsig10 may be used for tagging cells that express zsig10; for isolating zsig10 by affinity purification; for diagnostic assays for determining circulating levels of zsig10 polypeptides; for detecting or quantitating soluble zsig10 as marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block zsig10 polypeptide adhesion modulating or anti-microbial or like activity *in vitro* and *in vivo*. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Moreover, antibodies to zsig10 or fragments thereof may be used *in vitro* to

detect denatured zsig10 or fragments thereof in assays, for example, Western Blots or other assays known in the art.

Antibodies or polypeptides herein can also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. For instance, polypeptides or antibodies of the present invention can be used to identify or treat tissues or organs that express a corresponding anti-complementary molecule (receptor or antigen, respectively, for instance). More specifically, zsig10 polypeptides or anti-zsig10 antibodies, or bioactive fragments or portions thereof, can be coupled to detectable or cytotoxic molecules and delivered to a mammal having cells, tissues or organs that express the anti-complementary molecule.

Suitable detectable molecules may be directly or indirectly attached to the polypeptide or antibody, and include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. Suitable cytotoxic molecules may be directly or indirectly attached to the polypeptide or antibody, and include bacterial or plant toxins (for instance, diphtheria toxin, *Pseudomonas* exotoxin, ricin, abrin and the like), as well as therapeutic radionuclides, such as iodine-131, rhenium-188 or yttrium-90 (either directly attached to the polypeptide or antibody, or indirectly attached through means of a chelating moiety, for instance). Polypeptides or antibodies may also be conjugated to cytotoxic drugs, such as adriamycin. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/ anticomplementary pair, where the other member is bound to the polypeptide or antibody portion. For these purposes,

biotin/streptavidin is an exemplary complementary/ anticomplementary pair.

In another embodiment, polypeptide-toxin fusion proteins or antibody-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for instance, to treat cancer cells or tissues). Alternatively, if the polypeptide has multiple functional domains (i.e., an activation domain or a ligand binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule or a complementary molecule to a cell or tissue type of interest. In instances where the domain only fusion protein includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule fusion proteins thus represent a generic targeting vehicle for cell/tissue-specific delivery of generic anti-complementary-detectable/ cytotoxic molecule conjugates. The bioactive polypeptide or antibody conjugates described herein can be delivered intravenously, intraarterially, intraductally with DMSO, intramuscularly, subcutaneously, intraperitoneally, also by transdermal methods, by electro-transfer, orally or via inhalant.

Molecules of the present invention can be used to identify and isolate receptors involved in adherence. For example, proteins and peptides of the present invention can be immobilized on a column and membrane preparations run over the column (Immobilized Affinity Ligand Techniques, Hermanson et al., eds., Academic Press, San Diego, CA, 1992, pp.195-202). Proteins and peptides can also be radiolabeled (Methods in Enzymol., vol. 182, "Guide to Protein Purification", M. Deutscher, ed., Acad. Press, San Diego, 1990, 721-737) or photoaffinity labeled (Brunner et al., Ann. Rev. Biochem. 62:483-514, 1993 and

Fedan et al., Biochem. Pharmacol. 33:1167-80, 1984) and specific cell-surface proteins can be identified.

For pharmaceutical use, the proteins of the present invention are formulated for parenteral, particularly intravenous or subcutaneous, delivery according to conventional methods. Intravenous administration will be by bolus injection or infusion over a typical period of one to several hours. In general, pharmaceutical formulations will include a zsig10 protein in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc. Methods of formulation are well known in the art and are disclosed, for example, in Remington: The Science and Practice of Pharmacy, Gennaro, ed., Mack Publishing Co., Easton, PA, 19th ed., 1995. Therapeutic doses will generally be determined by the clinician according to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, etc. Determination of dose is within the level of ordinary skill in the art. The proteins may be administered for acute treatment, over one week or less, often over a period of one to three days or may be used in chronic treatment, over several months or years.

Polynucleotides encoding zsig10 polypeptides are useful within gene therapy applications where it is desired to increase or inhibit zsig10 activity. If a mammal has a mutated or absent zsig10 gene, the zsig10 gene can be introduced into the cells of the mammal. In one embodiment, a gene encoding a zsig10 polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus,

adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes simplex virus 1 (HSV1) vector (Kaplitt et al., Molec. Cell. Neurosci. 2:320-30, 1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., J. Clin. Invest. 90:626-30, 1992; and a defective adeno-associated virus vector (Samulski et al., J. Virol. 61:3096-101, 1987; Samulski et al., J. Virol. 63:3822-8, 1989).

In another embodiment, a zsig10 gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al. Cell 33:153, 1983; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., J. Virol. 62:1120, 1988; Temin et al., U.S. Patent No. 5,124,263; WIPO Publication WO 95/07358; and Kuo et al., Blood 82:845, 1993. Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7, 1987; Mackey et al., Proc. Natl. Acad. Sci. USA 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. More particularly, directing transfection to particular cells represents one area of benefit. For instance, directing transfection to particular cell types would be particularly advantageous in a tissue with cellular

heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., J. Biol. Chem. 267:963-7, 1992; Wu et al., J. Biol. Chem. 263:14621-4, 1988.

Antisense methodology can be used to inhibit zsig10 gene transcription, such as to inhibit cell proliferation *in vivo*. Polynucleotides that are complementary to a segment of a zsig10-encoding polynucleotide (e.g., a polynucleotide as set froth in SEQ ID NO:1) are designed to bind to zsig10-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of zsig10 polypeptide-encoding genes in cell culture or in a subject.

Transgenic mice, engineered to express the zsig10 gene, and mice that exhibit a complete absence of zsig10 gene function, referred to as "knockout mice" (Snouwaert et al., Science 257:1083, 1992), may also be generated (Lowell et al., Nature 366:740-42, 1993). These mice may be employed to study the zsig10 gene and the protein encoded thereby in an in vivo system.

The present invention also provides reagents for use in diagnostic applications. For example, the zsig10

gene, a probe comprising zsig10 DNA or RNA, or a subsequence thereof can be used to determine if the zsig10 gene is present on chromosome 7 or if a mutation has occurred. Detectable chromosomal aberrations at the zsig10 gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. These aberrations can occur within the coding sequence, within introns, or within flanking sequences, including upstream promoter and regulatory regions, and may be manifested as physical alterations within a coding sequence or changes in gene expression level.

In general, these diagnostic methods comprise the steps of (a) obtaining a genetic sample from a patient; (b) incubating the genetic sample with a polynucleotide probe or primer as disclosed above, under conditions wherein the polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first reaction product; and (iii) comparing the first reaction product to a control reaction product. A difference between the first reaction product and the control reaction product is indicative of a genetic abnormality in the patient. Genetic samples for use within the present invention include genomic DNA, cDNA, and RNA. The polynucleotide probe or primer can be RNA or DNA, and will comprise a portion of SEQ ID NO:1, the complement of SEQ ID NO:1, or an RNA equivalent thereof. Suitable assay methods in this regard include molecular genetic techniques known to those in the art, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, ligation chain reaction (Barany, PCR Methods and Applications 1:5-16, 1991), ribonuclease protection assays, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; Marian, Chest 108:255-65, 1995). Ribonuclease protection assays

(see, e.g., Ausubel et al., ibid., ch. 4) comprise the hybridization of an RNA probe to a patient RNA sample, after which the reaction product (RNA-RNA hybrid) is exposed to RNase. Hybridized regions of the RNA are protected from digestion. Within PCR assays, a patient's genetic sample is incubated with a pair of polynucleotide primers, and the region between the primers is amplified and recovered. Changes in size or amount of recovered product are indicative of mutations in the patient. Another PCR-based technique that can be employed is single strand conformational polymorphism (SSCP) analysis (Hayashi, PCR Methods and Applications 1:34-8, 1991).

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Extension of EST Sequence

The novel zsig10 polypeptide-encoding polynucleotides of the present invention were initially identified by querying an EST database for secretory signal sequences characterized by an upstream methionine start site, a hydrophobic region of approximately 13 amino acids and a cleavage site (SEQ ID NO: 5, wherein cleavage occurs between the alanine and arginine amino acid residues) in an effort to select for secreted proteins. Polypeptides corresponding to ESTs meeting those search criteria were compared to known sequences to identify secreted proteins having homology to known ligands. A single EST sequence was discovered and predicted to be related to secreted proteins found in *Xenopus laevis*. See, for example, Sive et al., Dev. Dyn. 205(3): 265-80 (1996) and Sive et al., Cell 58(1): 171-80 (1989). To identify the corresponding cDNA, a clone considered likely

to contain the entire coding region was used for sequencing. Using an Invitrogen S.N.A.P.™ Miniprep kit (Invitrogen, Corp., San Diego, CA) according to manufacturer's instructions a 5 ml overnight culture in LB + 50 µg/ml ampicillin was prepared. The template was sequenced on an ABIPRISM™ model 377 DNA sequencer (Perkin-Elmer Cetus, Norwalk, Ct.) using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corp.) according to manufacturer's instructions. Oligonucleotides ZC976 (SEQ ID NO: 9), ZC694 (SEQ ID NO: 10) and ZC6768 (SEQ ID NO: 11) to the LacZ, T7 and T3 promoters on the clone-containing vector were used as sequencing primers. Oligonucleotides ZC11668 (SEQ ID NO: 6), ZC12253 (SEQ ID NO: 7) and ZC12241 (SEQ ID NO: 8) were used to complete the sequence from the clone. Sequencing reactions were carried out in a Hybaid OmniGene Temperature Cycling System (National Labnet Co., Woodbridge, NY). SEQUENCHER™ 3.0 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI) was used for data analysis. The resulting 881 bp sequence is disclosed in SEQ ID NO: 1. Comparison of the originally derived EST sequence with the sequence represented in SEQ ID NO: 1 showed that there were 13 base pair differences and 9 base pair insertions which resulted in 10 amino acid differences and 9 frame shifts between the deduced amino acid sequences. Note that these numbers include base pair changes from unknown residues in the EST sequence to known residues in SEQ ID NO: 1, which result in "assumed" amino acid changes.

Example 2

Tissue Distribution

Northerns were performed using Human Multiple Tissue Blots from Clontech (Palo Alto, CA). A 40 bp DNA probe (ZC11668; SEQ ID NO: 6) to the 5' end of the

oligonucleotide sequence of the mature protein shown in SEQ ID NO: 1 was radioactively labeled with ^{32}P using T4 polynucleotide kinase and forward reaction buffer (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's specifications. The probe was purified using a NUCTRAP push column (Stratagene Cloning Systems, La Jolla, CA). EXPRESSHYB (Clontech, Palo Alto, CA) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place overnight at 42° C, and the blots were then washed in 2X SSC and 0.05% SDS at RT, followed by a wash in 1X SSC and 0.1% SDS at 60°C. Two transcript sizes were observed, one at approximately 1 kb and one at approximately 2 kb. The 1 kb message was detected in much higher abundance than the 2 kb message, with the 1 kb message expressed at least about 50 times higher in most tissues except trachea where the expression appeared to be approximately 25 times higher. Signal intensity was highest for lung, prostate, small intestine, colon, trachea and stomach, with relatively less intense signals in uterus, pancreas and kidney.

Example 3
Chromosomal Assignment and Placement of the zsig10 Gene

The zsig10 gene was mapped to chromosome 7 using the commercially available version of the Whitehead Institute/MIT Center for Genome Research's GeneBridge 4 Radiation Hybrid Panel (Research Genetics, Inc., Huntsville, AL). The GeneBridge 4 Radiation Hybrid Panel contained PCRable DNAs from each of 93 radiation hybrid clones, plus two control DNAs (the HFL donor and the A23 recipient). A publicly available WWW server (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) allowed for mapping relative to the Whitehead Institute/MIT Center for Genome Research's radiation hybrid map of the human genome

(the "WICGR" radiation hybrid map) which was constructed with the GeneBridge 4 Radiation Hybrid Panel.

For the mapping of the zsig10 gene with the GeneBridge 4 Radiation Hybrid Panel, 25 μl reactions were set up in a PCRable 96-well microtiter plate (Stratagene, La Jolla, CA) and used in a RoboCycler Gradient 96 thermal cycler (Stratagene). Each of the 95 PCR reactions consisted of 2.5 μl 10X KlenTaq PCR reaction buffer (CLONTECH Laboratories, Inc., Palo Alto, CA), 2 μl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1.25 μl sense primer, ZC 13173 (SEQ ID NO: 11), 1.25 μl antisense primer, ZC 13172 (SEQ ID NO: 12), 2.5 μl *Redi*Load (Research Genetics, Inc., Huntsville, AL), 0.5 μl 50X ADVANTAGE™ KlenTaq Polymerase Mix (Clontech Laboratories, Inc.), 25 ng of DNA from an individual hybrid clone or control and x μl ddH2O for a total volume of 25 μl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as follows: an initial 1 cycle 5 minute denaturation at 95°C, 35 cycles of a 1 minute denaturation at 95°C, 1 minute annealing at 64°C and 1 minute and 15 second extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 3% NuSieve® GTG agarose gel (FMC Bioproducts, Rockland, ME).

The results showed that the zsig10 gene maps 59.99 cR from the top of the human chromosome 7 linkage group on the WICGR radiation hybrid map. Relative to the centromere, its nearest proximal marker was AFM144ZA1 and its nearest distal maker was WI-11644. The use of surrounding markers positioned the zsig10 gene in the 7p21.1-p15.3 region on the integrated LDB chromosome 7 map (The Genetic Location Database, University of Southhampton, WWW server: http://cedar.genetics.soton.ac.uk/public_html/).

Example 4
Creation of mammalian expression vectors zsig10NF/pZP9, zsig10CF/pZP9 and zsig10/pZP9

Three expression vectors were prepared for the zsig10 polypeptide, zSIG10CF/pZP9 and zSIG10NF/pZP9, wherein the constructs are designed to express a zsig25 polypeptide with a C- or N-terminal FLAG tag (SEQ ID NO:35) and zSIG10/pZP9 expressing untagged zsig10 polypeptides.

ZSIG10/pZP9

A approximately 875 bp restriction digest fragment of ZSIG-10 DNA was derived from the clone described in Example 1 above. Five micrograms of the clone was digested with 1 µl each of the restriction enzymes Eco RI and Xho I. The resultant ligation fragment was then run on a 0.8% LMP agarose gel (Seaplaque GTG) with 0.5x TBE buffer. A band of the predicted size was excised and the DNA was purified from the gel with a QIAQUICK® column (Qiagen) according the manufacturer's instructions.

The excised, restriction digested zsig10 DNA was subcloned into plasmid pZP9 which had been cut with Eco RI and Xho I. Plasmid pZP9 (deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, multiple restriction sites for insertion of coding sequences, a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

zSIG10CF/pZP9

A 533 bp PCR generated ZSIG-10 DNA fragment was created using ZC13436 (SEQ ID NO:36) and ZC13435 (SEQ ID

NO:37) as PCR primers and the template described in Example 1 above. The PCR reaction was incubated at 94°C for 5 minutes, and then run for 10 cycles of 30 seconds at 94°C and 2 minutes at 75°C, followed by 15 cycles at 94°C for 30 seconds and 62°C for 2 minutes. The resultant PCR product was then run on a 0.9% GTG/TBE agarose gel with 1x TBE buffer. A band of the predicted size was excised and the DNA was purified from the gel with a QIAQUICK® column (Qiagen) according the manufacturer's instructions. The DNA was digested with the restriction enzymes BAM HI (Boehringer Mannheim) and Eco RI (Gibco BRL), followed by phenol/chloroform/isoamyl alcohol extraction and ETOH/glycogen precipitated.

The excised, restriction digested zsig10 DNA was subcloned into plasmid CF/pZP9 which had been cut with Eco RI and Bam HI. The zSIG10/CFpZP9 expression vector uses the native zSIG10 signal peptide, and the FLAG epitope (SEQ ID NO:35) is attached at the C-terminus as a purification aid. Plasmid CF/pZP9 (deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, multiple restriction sites for insertion of coding sequences, a sequence encoding the FLAG tag (SEQ ID NO:35), a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

zSIG10NF/pZP9

A 474 bp PCR generated zSIG10/NF DNA fragment was created in accordance with the procedure set forth above using Z13441 (SEQ ID NO:38) and ZC13442 (SEQ ID NO:39) as PCR primers. The purified PCR fragment was digested with the restriction enzymes Bam HI (Boehringer

Mannheim) and Xho I (Gibco BRL), followed by phenol/chloroform/isoamyl alcohol extraction and ETOH/glycogen precipitation.

The excised and restriction digested zSIG10 DNA was subcloned into plasmid NF/pZP9 which had been cut with Bam HI and Xho I. The zSIG10/NFpZP9 expression vector incorporates the TPA leader and attaches the FLAG tag (SEQ ID NO:35) to the N-terminal of the zsig10 polypeptide-encoding polynucleotide sequence. Plasmid NF/pZP9 (deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, a TPA leader peptide followed by the sequence encoding the FLAG tag (SEQ ID NO:35), multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also contains an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

For the untagged zsig10 construct, approximately 100 ng of the zsig10 insert and 100 ng of the Eco RI/Not I digested pZP9 vector were ligated as described for the tagged constructs. For the N- and C-tagged constructs, about 10 ng of the restriction digested inserts and 10 ng of the corresponding vectors were ligated at room temperature for 4 hours. One microliter of each ligation reaction was independently electroporated into DH10B competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 50 mg/ml ampicillin, and incubated overnight. Colonies were screened by PCR as described above. For zsig10/pZP9 screens the primers were ZC6583 (SEQ ID NO:40) and ZC5020 (SEQ ID NO:41), for zSIG10CF/pZP9 screens the primers were, ZC13435 (SEQ ID NO: 37) and ZC13436 (SEQ ID NO:36) and for zSIG10NF/pZP9

screens the primers were ZC13442 (SEQ ID NO:39) and ZC13441 (SEQ ID NO:38). The insert sequence of positive clones, 950 bp for zsig10 untagged, 474 bp fragment for zSIG10NF and a 533 bp fragment for zSIG10/CF were verified by sequence analysis. A large scale plasmid preparation was done using a QIAGEN® Maxi prep kit (Qiagen) according to manufacturer's instructions.

Example 5
Expression of zsig10NF/pZP9, zsig10CF/pZP9 and zsig10/pZP9

BHK 570 cells (ATCC NO: CRL-10314) were plated in 10 cm tissue culture dishes and allowed to grow to approximately 50 to 70% confluency overnight at 37°C, 5% CO_2, in DMEM/FBS media (DMEM, Gibco/BRL High Glucose, (Gibco BRL, Gaithersburg, MD), 5% fetal bovine serum (Hyclone, Logan, UT), 1 µM L-glutamine (JRH Biosciences, Lenexa, KS), 1 µM sodium pyruvate (Gibco BRL)). The cells were then transfected with the plasmid zsig10NF/pZP9 (N-terminal FLAG tag), zsig10CF/pZP9 (C-terminal FLAG tag), or zsig10/pZP9 (untagged), using Lipofectamine™ (Gibco BRL), in serum free (SF) media formulation (DMEM, 10 mg/ml transferrin, 5 mg/ml insulin, 2 mg/ml fetuin, 1% L-glutamine and 1% sodium pyruvate). Sixteen micrograms of zsig10NF/pZP9, 16 µg of zsig10CF/pZP9 and 16 µg zsig10/pZP9 were separately diluted into 15 ml tubes to a total final volume of 640 µl with SF media. In separate tubes, 35 µl of Lipofectamine™ (Gibco BRL) was mixed with 605 µl of SF medium. The Lipofectamine™ mix was added to the DNA mix and allowed to incubate approximately 30 minutes at room temperature. Five milliliters of SF media was added to the DNA:Lipofectamine™ mixture. Three plates of cells were rinsed once with 5 ml of SF media, aspirated, and the DNA:Lipofectamine™ mixture was added. The cells were incubated at 37°C for five hours, then 6.4 ml of DMEM/10% FBS, 1% PSN media was added to each plate. The plates were incubated at 37°C overnight and the

DNA:Lipofectamine™ mixture was replaced with fresh 5% FBS/DMEM media the next day. On day 2 post-transfection, the cells were split into the selection media (DMEM/FBS media from above with the addition of 1 μM methotrexate (Sigma Chemical Co., St. Louis, Mo.)) in 150 mm plates at 1:10, 1:20 and 1:50. The cells were refed at day 5 post-transfection with fresh selection media. Approximately 10 days post-transfection, two 150 mm culture dishes of methotrexate resistant colonies from each transfection were trypsinized and the cells were pooled and plated into a T-162 flask and transferred to large scale culture.

Example 6

Large Scale Culture of zsig10 FLAG-tagged and untagged polypeptides

One T-162 flask, containing confluent cells expressing zsig10/NF and one containing confluent cells expressing zsig10-untagged, obtained from the expression procedure described above, were expanded into six T-162 flasks. One of the six resulting flasks was used to freeze down four cryovials, and the other five flasks were used to generate a Nunc cell factory.

The cells from the five T-165 flasks were used to seed a Nunc cell factory (10 layers, commercially available from VWR). Briefly, the cells from the T-162 flasks described above containing cells expressing zsig10-NF were detached using trypsin, pooled, and added to 1.5 liters ESTEP1 media (668.7g/50L DMEM (Gibco), 5.5 g/50L pyruvic acid, sodium salt 96% (Mallinckrodt), 185.0 g/50L $NaHCO_3$ (Mallinkrodt), 5.0 mg/ml and 25 ml/50L insulin (JRH Biosciences), 10.0 mg/ml and 25 ml/50L transferrin (JRH Biosciences), 2.5L/50L fetal bovine serum (characterized) (Hyclone), 1 μM MTX, with pH adjusted to 7.05 +/- 0.05) prewarmed to 37°C. The cells from the T-162 flasks described above containing cells expressing untagged zsig10 were detached using trypsin, pooled, and added to

1.5 liters of SL6V2 media (13.3 g/l DMEM, 0.11 g/l Na-pyruvate, 3.7 g/l $NaHCO_3$, 5.96 g/l HEPES (JRH Biosciences, Lenexa, KS) and 50 ml/l FBS (Hyclone, Logan, UT), pH 7.05). The media containing cells was then poured into Nunc cell factories via a funnel. The cell factories were placed in a 37°C/5.0% CO2 incubator.

At 80-100% confluence, a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Since no contamination was observed, supernatant from the confluent factories was poured into a small harvest container, sampled and discarded. The adherent cells were then washed once with 400 ml PBS. To detach the cells from the factories, 100 mls of trypsin was added to each and removed and the cells were then incubated for 5 to 10 minutes in the residual trypsin. The zsig10NF cells were collected following two, 200 ml washes of ESTEP1 media, the untagged zsig10 cells were collected in ESTEP Form, 5%HIA-FBS/DMEM media. For each construct, 40 ml of collected cells were then used to seed each of ten Nunc cell factories. For zsig10-NF cells, to each of ten ESTEP1 media-containing bottles (1.5 liters each, at 37°C) was added 40 mls of collected cells. One 1.5 liter bottle was then used to fill one Nunc factory. For untagged zsig10 cells, ESTEP FORM. 5%FBS/DMEM media was used. Each cell factory was placed in a 37°C/5.0% CO_2 incubator.

At 80-90% confluence, a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Since no contamination was observed, supernatant from the confluent factories were poured into a small harvest container, sampled and discarded. Cells were then washed once with 400 ml PBS. To the factories containing zsig10-NF cells, 1.5 liters of ESTEP2 media (668.7g/50L DMEM (Gibco), 5.5 g/50L pyruvic acid, sodium salt 96% (Mallinckrodt), 185.0 g/50L $NaHCO_3$ (Mallinkrodt), 5.0 mg/ml, 25 ml/50L insulin, 10.0 mg/ml and 25 ml/50L

transferrin) was added to each Nunc cell factory. To factories containing untagged zsig10 cells, 1.5 liters of serum free ESTEP FORM. media was added. The cell factories were incubated at 37°C/5.0% CO_2.

At approximately 76 hours (zsig10/NF, 15L was obtained) and 65 hours (untagged zsig10, 15L was obtained), a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Supernatant from each factory was poured into small harvest containers. Fresh serum-free media (1.5 liters) was poured into each Nunc cell factory, and the factories were incubated at 37°C/5.0% CO_2. One ml of supernatant harvest was transferred to a microscope slide, and subjected to microscopic analysis for contamination. The contents of the small harvest containers for each factory were pooled and immediately filtered. A second harvest was then performed, substantially as described above at 40 hours (zsig10/NF, 15 L were obtained) and 52 hours (untagged zsig10, 15 L were obtained) and the cell factories were discarded thereafter. An aseptically assembled filter train apparatus was used for aseptic filtration of the harvest supernatant (conditioned media). Assembly was a follows: tubing was wire-tied to an Opti-Cap filter (Millipore Corp., Bedford, MA) and a Gelman Supercap 50 filter (Gelman Sciences, Ann Arbor, MI). The Supercap 50 filter was also attached to a sterile capped container located in a hood; tubing located upstream of the Millipore Opti-cap filter was inserted into a peristaltic pump; and the free end of the tubing was placed in the large harvest container. The peristaltic pump was run between 200 and 300 rpm, until all of the conditioned media passed through the 0.22 µm final filter into a sterile collection container. The filtrate was placed in a 4 °C cold room pending purification.

Conditioned media containing zsig10/NF and untagged zsig10 was collected for concentration at various

time points (at the 5 T-162 flask stage; 1 factory, fetal bovine serum media; 10 factories, fetal bovine serum media; 10 factories, serum free media and a second 10 factory, serum free media time point). Since the expected mass of the protein was in excess of 8 kDA, Millipore 5 kDa cut off concentrators were used. The starting volume for each sample was 15 ml, which was concentrated to a final volume of 1.5 ml. The concentrators were spun at 4°C in Beckman tabletop centrifuge at 2000 x g (3000 rpm) for 40 minutes. The concentrate was transferred to a 1.5 ml non-stick microfuge tube, and the volume was adjusted to 1 ml using flow through media to achieve a 10x concentration. To sterilize the media, the 10x concentrate was split into two Costar Spin-X tubes, and the tubes were spun at 8000 x g for two minutes in a Eppendorf 5415 microfuge (VWR, Seattle, WA).

Example 7

Construction of ZSIG10 Amino Terminal Glu-Glu Tagged and Carboxy Terminal Glu-Glu Tagged Yeast Expression Vectors

Expression of zsig10 in *Pichia methanolica* utilizes the expression system described in co-assigned WIPO publication WO 97/17450. An expression plasmid containing all or part of a polynucleotide encoding zsig10 is constructed via homologous recombination. An expression vector was built from pCZR204 to express C-terminal Glu-Glu-tagged (CEE) zsig10 polypeptides. The pCZR204 vector contains the AUG1 promoter, followed by the αFpp leader sequence, followed by a blunt-ended Sma I restriction site, a carboxy-terminal peptide tag (Glu-Glu), a translational STOP codon, followed by the AUG1 terminator, the ADE2 selectable marker, and finally the AUG1 3' untranslated region. Also included in this vector are the URA3 and CEN-ARS sequences required for selection and replication in *S. cerevisiae*, and the AmpR and colE1

ori sequences required for selection and replication in *E. coli*. A second expression vector was built from zCZR191 to express a N-terminal Glu-Glu-tagged (NEE) zsig10 polypeptides. The zCZR191 expression vector is as described above, having an amino terminal Glu-Glu tag. The zsig10 sequence inserted into these vectors begins at residue 21 (Arg) of the zsig10 amino acid sequence (SEQ ID NO:2).

For each construct two linkers are prepared, and along with zsig10, were homologously recombined into the yeast expression vectors described above. The untagged N-terminal linker (SEQ ID NO:29) spans 70 base pairs of the alpha factor prepro (aFpp) coding sequence on one end and joins it to the 70 base pairs of the amino-terminus coding sequence from the mature zsig10 sequence on the other. The NEE-tagged linker (SEQ ID NO:19) joins Glu-Glu tag (SEQ ID NO:42) between the aFpp coding sequence and the zsig10 sequence. The untagged C-terminal linker (SEQ ID NO:24) spans about 70 base pairs of carboxy terminus coding sequence of the zsig10 on one end with 70 base pairs of AUG1 terminator sequence. The CEE-tagged linker (SEQ ID NO:34) inserts the Glu-Glu tag (SEQ ID NO:42) between the C-terminal end of zsig10 and the AUG1 terminator region.

Construction of the NEE-tagged-Zsig10 plasmid

An NEE-tagged-zsig10 plasmid was made by homologously recombining 100 ng of the SmaI digested pCZR190 acceptor vector, 1 µg of Eco RI-Xho I zsig10 cDNA donor fragment, 1 µg NEE-tagged-zsig10 linker (SEQ ID NO:19) and 1 µg of C-terminal untagged linker (SEQ ID NO:24) in *S. cerevisiae*.

The NEE-zsig10 linker was synthesized by a PCR reaction. To a final reaction volume of 100 µl was added 1 pmol each of linkers, ZC13,731 (SEQ ID NO:16) and ZC13,729 (SEQ ID NO:17), and 100 pmol of each primer ZC13,497 (SEQ ID NO:15) and ZC13,730 (SEQ ID NO:18), 10 µl

of 10X PCR buffer (Boehringer Mannheim), 1 µl Pwo Polymerase (Boehringer Mannheim), 10 µl of 0.25 mM nucleotide triphosphate mix (Perkin Elmer) and dH_2O. The PCR reaction was run 10 cycles at 30 seconds at 94°C, 1 minute at 50°C and 1 minute at 72°C, concluded with a 6 minute extension at 72°. The resulting 141 bp double stranded, NEE-tagged linker is disclosed in SEQ ID NO:19.

The C-terminal untagged zsig10 linker was made via a PCR reaction as described using oligonucleotides ZC13,734 (SEQ ID NO:23), ZC13,732 (SEQ ID NO:20), ZC13,728 (SEQ ID NO:21) and ZC13,733 (SEQ ID NO:22). The resulting 129 bp double stranded, C-terminal untagged linker is disclosed in SEQ ID NO:24.

Construction of the CEE-zsig10 plasmid

A CEE-zsig10 plasmid was made by homologously recombining 100 ng of Sma I digested pCZR204 acceptor vector, the 1µg of Eco RI-Xho I zsig10 cDNA donor fragment, 1 µg of N-terminal untagged zsig10 linker (SEQ ID NO:29) and 1 µg of CEE-tagged linker (SEQ ID NO:34) in a *S. cerevisiae*.

The N-terminal untagged zsig10 linker was made via a PCR reaction as described above using oligonucleotides ZC14,822 (SEQ ID NO:25), ZC14,821 (SEQ ID NO:26), ZC14,832 (SEQ ID NO:27) and ZC14,833 (SEQ ID NO:28). The resulting 147 bp double stranded, N-terminal untagged linker is disclosed in SEQ ID NO:29.

The CEE-tagged linker was made via a PCR reaction as described above using ZC14,834 (SEQ ID NO:30), ZC15,957 (SEQ ID NO:31), ZC15,632 (SEQ ID NO:32) and ZC 14,820 (SEQ ID NO:33). The resulting approximately 1145 bp double stranded, CEE-tagged linker is disclosed in SEQ ID NO:34.

One hundred microliters of competent yeast cells (*S. cerevisiae*) was independently combined with 10 µl of the various DNA mixtures from above and transferred to a

0.2 cm electroporation cuvette. The yeast/DNA mixtures were electropulsed at 0.75 kV (5 kV/cm), ∞ ohms, 25 μF. To each cuvette was added 600 μl of 1.2 M sorbitol and the yeast was plated in two 300 μl aliquots onto two URA D plates and incubated at 30°C.

After about 48 hours the Ura^+ yeast transformants from a single plate were resuspended in 2.5 ml H_2O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 1 ml of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). Five hundred microliters of the lysis mixture was added to an Eppendorf tube containing 300 μl acid washed glass beads and 200 μl phenol-chloroform, vortexed for 1 minute intervals two or three times, followed by a 5 minute spin in a Eppendorf centrifuge as maximum speed. Three hundred microliters of the aqueous phase was transferred to a fresh tube and the DNA precipitated with 600 μl ethanol (EtOH), followed by centrifugation for 10 minutes at 4°C. The DNA pellet was resuspended in 100 μl H_2O.

Transformation of electrocompetent *E. coli* cells (DH10B, Gibco BRL) was done with 0.5-2 μl yeast DNA prep and 40 ul of DH10B cells. The cells were electropulsed at 2.0 kV, 25 μF and 400 ohms. Following electroporation, 1 ml SOC (2% Bacto™ Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) was plated in 250 μl aliquots on four LB AMP plates (LB broth (Lennox), 1.8% Bacto™ Agar (Difco), 100 mg/L Ampicillin).

Individual clones harboring the correct expression construct for NEE tagged zsig10 were identified by restriction digest to verify the presence of the zsig10 insert and to confirm that the various DNA sequences had been joined correctly to one another. For CEE-tagged zsig10, correct expression constructs were identified by PCR as described above using oligos ZC14834 (SEQ ID NO:30) and ZC14820 (SEQ ID NO:33) which gave a 145 bp fragment

and oligos 14822 (SEQ ID NO:25) and ZC14833 (SEQ ID NO:28) which gave a 147 bp fragment. The insert of positive clones were subjected to sequence analysis. Larger scale plasmid DNA was isolated using the Qiagen Maxi kit (Qiagen) according to manufacturer's instruction and the DNA was digested with Not I to liberate the *Pichia*-Zsig10 expression cassette from the vector backbone. The Not I-restriction digested DNA fragment was then transformed into the *Pichia methanolica* expression host, PMAD16. This was done by mixing 100 µl of prepared competent PMAD16 cells with 10 µg of Not I restriction digested zsig10 and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV, 25 µF, infinite ohms. To the cuvette was added 1 ml of 1X Yeast Nitrogen Base and 500 µl aliquots were plated onto two ADE DS (0.056% -Ade -Trp -Thr powder, 0.67% yeast nitrogen base without amino acids, 2% D-glucose, 0.5% 200X tryptophan, threonine solution, and 18.22% D-sorbitol) plates for selection and incubated at 30°C. The resulting NEE-tagged-zsig10 plasmid containing yeast cells were designated PMAD16::pSDH112-5 and the CEE-tagged-zsig10 plasmid containing yeast cells were designated PMAD16::pTAP13. The transformed yeast cells were plated on ADE DS plates for selection. Clones were picked and screened via Western blot for high-level Zsig10 expression and subjected to fermentation.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: ZymoGenetics, Inc.
1201 Eastlake Ave. E
Seattle
Washington
USA
98102

(ii) TITLE OF THE INVENTION: SECRETED ZSIG10 POLYPEPTIDES

(iii) NUMBER OF SEQUENCES: 42

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ZymoGenetics
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(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98102

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:60/039,631
(B) FILING DATE: March 19, 1997

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(B) REGISTRATION NUMBER: 41,156
(C) REFERENCE/DOCKET NUMBER: 97-06PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 206-442-6675

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(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 881 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 63...587
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GAATTCGGCA CGAGAGCCGC CGACTCACAC AAGGCAGGTG GGTGAGGAAA TCCAGAGTTG      60
CC ATG GAG AAA ATT CCA GTG TCA GCA TTC TTG CTC CTT GTG GCC CTC        107
   Met Glu Lys Ile Pro Val Ser Ala Phe Leu Leu Leu Val Ala Leu
    1               5                  10                  15

TCC TAC ACT CTG GCC AGA GAT ACC ACA GTC AAA CCT GGA GCC AAA AAG       155
Ser Tyr Thr Leu Ala Arg Asp Thr Thr Val Lys Pro Gly Ala Lys Lys
                20                  25                  30

GAC ACA AAG GAC TCT CGA CCC AAA CTG CCC CAG ACC CTC TCC AGA GGT       203
Asp Thr Lys Asp Ser Arg Pro Lys Leu Pro Gln Thr Leu Ser Arg Gly
            35                  40                  45

TGG GGT GAC CAA CTC ATC TGG ACT CAG ACA TAT GAA GAA GCT CTA TAT       251
Trp Gly Asp Gln Leu Ile Trp Thr Gln Thr Tyr Glu Glu Ala Leu Tyr
        50                  55                  60

AAA TCC AAG ACA AGC AAC AAA CCC TTG ATG ATT ATT CAT CAC TTG GAT       299
Lys Ser Lys Thr Ser Asn Lys Pro Leu Met Ile Ile His His Leu Asp
    65                  70                  75

GAG TGC CCA CAC AGT CAA GCT TTA AAG AAA GTG TTT GCT GAA AAT AAA       347
Glu Cys Pro His Ser Gln Ala Leu Lys Lys Val Phe Ala Glu Asn Lys
80                  85                  90                  95
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GAA ATC CAG AAA TTG GCA GAG CAG TTT GTC CTC CTC AAT CTG GTT TAT      395
Glu Ile Gln Lys Leu Ala Glu Gln Phe Val Leu Leu Asn Leu Val Tyr
                100                 105                 110

GAA ACA ACT GAC AAA CAC CTT TCT CCT GAT GGC CAG TAT GTC CCC AGG      443
Glu Thr Thr Asp Lys His Leu Ser Pro Asp Gly Gln Tyr Val Pro Arg
            115                 120                 125

ATT ATG TTT GTT GAC CCA TCT CTG ACA GTT AGA GCC GAT ATC ACT GGA      491
Ile Met Phe Val Asp Pro Ser Leu Thr Val Arg Ala Asp Ile Thr Gly
        130                 135                 140

AGA TAT TCA AAT CGT CTC TAT GCT TAC GAA CCT GCA GAT ACA GCT CTG      539
Arg Tyr Ser Asn Arg Leu Tyr Ala Tyr Glu Pro Ala Asp Thr Ala Leu
    145                 150                 155

TTG CTT GAC AAC ATG AAG AAA GCT CTC AAG TTG CTG AAG ACT GAA TTG T    588
Leu Leu Asp Asn Met Lys Lys Ala Leu Lys Leu Leu Lys Thr Glu Leu
160                 165                 170                 175

AAAGAAAAAA AATCTCCAAG CCCTTCTGTC TGTCAGGCCT TGAGACTTGA AACCAGAAGA    648
AGTGTGAGAA GACTGGCTAG TGTGGAAGCA TAGTGAACAC ACTGATTAGG TTATGGTTTA    708
ATGTTACAAC AACTATTTTT TAAGAAAAAC AAGTTTTAGA AATTTGGTTT CAAGTGTACA    768
TGTGTGAAAA CAATATTGTA TACTACCATA GTGAGCCATG ATTTTCTAAA AAAAAAAATA    828
AATGTTTTGG GGGTGTTCTG TTTTCTCCAA AAAAAAAAAA AAAAAAACTC GAG           881
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Lys Ile Pro Val Ser Ala Phe Leu Leu Leu Val Ala Leu Ser
1 5 10 15
Tyr Thr Leu Ala Arg Asp Thr Thr Val Lys Pro Gly Ala Lys Lys Asp
20 25 30
Thr Lys Asp Ser Arg Pro Lys Leu Pro Gln Thr Leu Ser Arg Gly Trp
35 40 45
Gly Asp Gln Leu Ile Trp Thr Gln Thr Tyr Glu Glu Ala Leu Tyr Lys
50 55 60
Ser Lys Thr Ser Asn Lys Pro Leu Met Ile Ile His His Leu Asp Glu
65 70 75 80
Cys Pro His Ser Gln Ala Leu Lys Lys Val Phe Ala Glu Asn Lys Glu
85 90 95
Ile Gln Lys Leu Ala Glu Gln Phe Val Leu Leu Asn Leu Val Tyr Glu
100 105 110
Thr Thr Asp Lys His Leu Ser Pro Asp Gly Gln Tyr Val Pro Arg Ile
115 120 125
Met Phe Val Asp Pro Ser Leu Thr Val Arg Ala Asp Ile Thr Gly Arg
130 135 140
Tyr Ser Asn Arg Leu Tyr Ala Tyr Glu Pro Ala Asp Thr Ala Leu Leu
145 150 155 160
Leu Asp Asn Met Lys Lys Ala Leu Lys Leu Leu Lys Thr Glu Leu
165 170 175

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 173 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gln Thr Gly Leu Ser Leu Ala Cys Leu Val Leu Leu Cys Ser Val
1 5 10 15
Leu Gly Glu Ala Ala Leu Arg Lys Pro Lys Arg Gln Ala Ala Ala Thr
20 25 30
Asp Thr Asn Gly Ala Ala Lys Ser Glu Pro Ala Pro Val Lys Thr Lys
35 40 45
Gly Leu Lys Thr Leu Asp Arg Gly Trp Gly Glu Asp Ile Glu Trp Ala
50 55 60
Gln Thr Tyr Glu Glu Gly Leu Ala Lys Ala Arg Glu Asn Asn Lys Pro
65 70 75 80

Leu Met Val Ile His His Leu Glu Asp Cys Pro Tyr Ser Ile Ala Leu
85 90 95
Lys Lys Ala Phe Val Ala Asp Lys Met Ala Gln Lys Leu Ala Gln Glu
100 105 110
Asp Phe Ile Met Leu Asn Leu Val His Pro Val Ala Asp Glu Asn Gln
115 120 125
Ser Pro Asp Gly His Tyr Val Pro Lys Gly Ile Phe Ile Asp Pro Ser
130 135 140
Leu Thr Val Arg Ser Asp Leu Lys Gly Arg Tyr Gly Asn Lys Leu Tyr
145 150 155 160
Ala Tyr Asp Ala Asp Asp Ile Pro Glu Leu Ile Thr Thr
165 170

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gln Ala Gly Leu Ser Leu Val Cys Leu Val Leu Leu Cys Ser Ala
1 5 10 15
Leu Gly Glu Ala Val Leu Lys Lys Pro Lys Lys Gln Ala Gly Thr Thr
20 25 30
Asp Thr Lys Thr Asp Gln Glu Pro Ala Pro Ile Lys Thr Lys Gly Leu
35 40 45
Lys Thr Leu Asp Arg Gly Trp Gly Glu Ser Ile Glu Trp Val Gln Thr
50 55 60
Tyr Glu Glu Gly Leu Ala Lys Ala Arg Glu Asn Asn Lys Pro Leu Met
65 70 75 80
Val Ile His His Leu Glu Asp Cys Pro Tyr Ser Ile Ala Leu Lys Lys
85 90 95
Ala Phe Val Ala Asp Arg Met Ala Gln Lys Leu Ala Gln Glu Asp Phe
100 105 110
Ile Met Leu Asn Leu Val His Pro Val Ala Asp Glu Asn Gln Ser Pro
115 120 125
Asp Gly His Tyr Val Pro Arg Val Ile Phe Ile Asp Pro Ser Leu Thr
130 135 140
Val Arg Ser Asp Leu Lys Gly Arg Tyr Gly Asn Lys Met Tyr Ala Tyr
145 150 155 160

Asp Ala Asp Asp Ile Pro Glu Leu Ile Thr Asn Met Lys Lys Ala Lys
                165   170   175
Ser Phe Leu Lys Thr Glu Leu
            180

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Phe Leu Leu Leu Val Ala Leu Ser Tyr Thr Leu Ala Arg Asp
1   5   10   15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC1168

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTGTCCTTTT TGGCTCCAGG TTTGACTGTG GTATCTCTGG 40

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC12253

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGAGGACAAA CTGCTCTGCC 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC12241

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTGTCCTC CTCAATCTGG 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC976

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGTTGTAAAA CGACGGCC 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC694

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TAATACGACT CACTATAGGG 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6768

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCAATTAACC CTCACTAAAG GGAAC 25

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13173

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AAATTCCAGT GTCAGCAT 18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13172

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGAGTCCTTT GTGTCCTT

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 525 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
ATGGARAARA THCCNGTNWS NGCNTTYYTN YTNYTNGTNG CNYTNWSNTA YACNYTNGCN    60
MGNGAYACNA CNGTNAARCC NGGNGCNAAR AARGAYACNA ARGAYWSNMG NCCNAARYTN   120
CCNCARACNY TNWSNMGNGG NTGGGGNGAY CARYTNATHT GGACNCARAC NTAYGARGAR   180
GCNYTNTAYA ARWSNAARAC NWSNAAYAAR CCNYTNATGA THATHCAYCA YYTNGAYGAR   240
TGYCCNCAYW SNCARGCNYT NAARAARGTN TTYGCNGARA AYAARGARAT HCARAARYTN   300
GCNGARCART TYGTNYTNYT NAAYYTNGTN TAYGARACNA CNGAYAARCA YYTNWSNCCN   360
GAYGGNCART AYGTNCCNMG NATHATGTTY GTNGAYCCNW SNYTNACNGT NMGNGCNGAY   420
ATHACNGGNM GNTAYWSNAA YMGNYTNTAY GCNTAYGARC CNGCNGAYAC NGCNYTNYTN   480
YTNGAYAAYA TGAARAARGC NYTNAARYTN YTNAARACNG ARYTN                   525
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC13497

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGCATTGCTG CTAAAGAAGA AGGTGTAAGC TTGGACAAGA GAGA 44

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13731

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGTAAGCT TGGACAAGAG AGAAGAAGAA TACATGCCAA TGGAAGGTGG T 51

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13729

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGTGTCCTTT TTGGCTCCAG GTTTGACTGT GGTATCTCTA CCACCTTCCA TTGGCATGTA 60
TTC 63

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13730

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGTCTGGGGC AGTTTGGGTC GAGAGTCCTT TGTGTCCTTT TTGGCTCCAG GTT 53

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AGCATTGCTG CTAAAGAAGA AGGTGTAAGC TTGGACAAGA GAGAAGAAGA ATACATGCCA 60
ATGGAAGGTG GTAGAGATAC CACAGTCAAA CCTGGAGCCA AAAAGGACAC AAAGGACTCT 120
CGACCCAAAC TGCCCCAGAC C 141

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13732

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCTGCAGATA CAGCTCTGTT GCTTGACAAC ATGAAGAAAG CTCTCAAGTT GCTG 54

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13728

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGAAGAAAG CTCTCAAGTT GCTGAAGACT GAATTGTAAT AGTATTCTAG GGCTGCCTGT 60
TTG 63

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13733

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGGCAAACTC TCAAAAATTA TAAAAATATC CAAACAGGCA GCCCTAGAAT ACTA 54

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13734

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATCATAGAAG AGAAAAACAT TAGTTGGCAA ACTCTCAAAA ATTATAAAAA TA 52

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
CCTGCAGATA CAGCTCTGTT GCTTGACAAC ATGAAGAAAG CTCTCAAGTT GCTGAAGACT     60
GAATTGTAAT AGTATTCTAG GGCTGCCTGT TTGGATATTT TTATAATTTT TGAGAGTTTG    120
CCAACTAATG TTTTTCTCTT CTATGAT                                        147
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC14822

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
ACGGTTTATT GTTTATCAAT ACTACTATTG CTAGCATTGC                           40
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC14821

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TCAATACTAC TATTGCTAGC ATTGCTGCTA AAGAAGAAGG TGTAAGCTTG GACAAGAGAG 60
AA 62

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC14832

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CCTTTGTGTC CTTTTTGGCT CCAGGTTTGA CTGTGGTATC TCTTTCTCTC TTGTCCAAGC 60
TTACACCT 68

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC14833

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGGTCTGGGG CAGTTTGGGT CGAGAGTCCT TTGTGTCCTT TTTGGCTCCA 50

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ACGGTTTATT GTTTATCAAT ACTACTATTG CTAGCATTGC TGCTAAAGAA GAAGGTGTAA 60
GCTTGGACAA GAGAGAAAGA GATACCACAG TCAAACCTGG AGCCAAAAAG GACACAAAGG 120
ACTCTCGACC CAAACTGCCC CAGACCC 147

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC14834

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ACGAACCTGC AGATACAGCT CTGTTGCTTG ACAACATGAA GAAAGCTC 48

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC15957

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGCTTGACAA CATGAAGAAA GCTCTCAAGT TGCTGAAGAC TGAATTGGGA GGCGAGGAGT 60
ATATGCCTA 69

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 58 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC15632

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AACAGGCAGC CCTAGAATAC TAGGAATTCT ACTCCATAGG CATATACTCC TCGCCTCC 58

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC14820

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ATTATAAAAA TATCCAAACA GGCAGCCCTA GAATACTAG 39

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 148 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACGAACCTGC AGATACAGCT CTGTTGCTTG ACAACATGAA GAAAGCTCTC AAGTTGCTGA 60
AGACTGAATT GGGAGGCGAG GAGTATATGC CTATGGAGTA GAATTCCTAG TATTCTAGGG 120
CTGCCTGTTG TTTGGATATT TTTATAAT 148

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13436

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCGCGAATTC ATGGAGAAAA TTCCA 25

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13435

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CGCGGGATCC CAATTCAGTC TTCAG 25

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13441

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GCGCGGATCC AGAGATACCA CAGTC 25

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13442

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGCGCTCGAG TTACAATTCA GTCTT 25

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6583

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CACTGGAGTG GCAACTTCCA G 21

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC5020

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GTCCAACGAC TATAAAGAGG G 21

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Glu Glu Tyr Met Pro Met Glu
1 5

CLAIMS

What is claimed is:

1. An isolated polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 21-175 of SEQ ID NO:2.

2. An isolated polypeptide according to claim 1, wherein said polypeptide is at least 90% identical in amino acid sequence to residues 21-175 of SEQ ID NO:2.

3. An isolated polypeptide according to claim 1, wherein said polypeptide further comprises a cysteine residue corresponding to amino acid residue 81 of SEQ ID NO:2.

4. An isolated polypeptide according to claim 1, wherein said polypeptide further comprises a copper binding site corresponding to amino acid residues 74-78 of SEQ ID NO:2.

5. An isolated polypeptide according to claim 1, wherein said polypeptide comprises residues 26-175 of SEQ ID NO:2.

6. An isolated polypeptide according to claim 2, wherein said polypeptide comprises residues 21-175 of SEQ ID NO:2.

7. An isolated polypeptide according to claim 1, wherein said polypeptide comprises residues 1-175 of SEQ ID NO:2.

8. An isolated polypeptide according to claim 1, wherein said polypeptide is at least 1 kb in length.

9. An isolated polypeptide according to claim 1, covalently linked to a moiety selected from the group consisting of affinity tags, toxins, radionucleotides, enzymes and fluorophores.

10. An isolated polypeptide according to claim 9, wherein said moiety is an affinity tag selected from the group consisting of polyhistidine, FLAG, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region.

11. An isolated polypeptide according to claim 10 further comprising a proteolytic cleavage site between said sequence of amino acid residues and said affinity tag.

12. A DNA construct encoding a polypeptide fusion, said fusion comprising a secretory signal sequence having the amino acid sequence of amino acid residues 1-20 of SEQ ID NO:2, wherein said secretory signal sequence is operably linked to an additional polypeptide.

13. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 21-175 of SEQ ID NO:2; and

a transcriptional terminator.

14. An expression vector according to claim 13, wherein said DNA segment encodes a polypeptide is at least 90% identical in amino acid sequence to residues 21-175 of SEQ ID NO:2.

15. An expression vector according to claim 13, wherein said DNA segment encodes a polypeptide further comprising a cysteine residue corresponding to amino acid residue 81 of SEQ ID NO:2.

16. An expression vector according to claim 13, wherein said DNA segment encodes a polypeptide further

comprising a cooper binding site corresponding to amino acid residues 74-78 of SEQ ID NO:2.

17. An expression vector according to claim 13, wherein said polypeptide encoded by said DNA segment comprises residues 26-175 of SEQ ID NO:2.

18. An expression vector according to claim 13, wherein said polypeptide encoded by said DNA segment comprises residues 21-175 of SEQ ID NO:2.

19. An expression vector according to claim 13, wherein said polypeptide encoded by said DNA segment comprises residues 1-175 of SEQ ID NO:2.

20. An expression vector according to claim 13, wherein said polypeptide encoded by said DNA segment is at least 1 kb in length.

21. An expression vector according to claim 13, wherein said DNA segment encodes a polypeptide covalently linked to an affinity tag selected from the group consisting of polyhistidine, FLAG, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region.

22. An expression vector according to claim 13 wherein said DNA further encodes a secretory signal sequence operably linked to said polypeptide.

23. An expression vector according the claim 22, wherein said DNA encodes the secretory signal sequence having the amino acid sequence of residues 1-20 of SEQ ID NO:2.

24. A cultured cell into which has been introduced an expression vector according to claim 13, wherein said cell expresses said polypeptide encoded by said DNA segment.

25. A method of producing a protein comprising:
culturing a cell into which has been introduced an expression vector according to claim 13, whereby said cell expresses said protein encoded by said DNA segment; and
recovering said expressed protein.

26. A pharmaceutical composition comprising a polypeptide according to claim 1 in combination with a pharmaceutically acceptable vehicle.

27. An antibody that specifically binds to an epitope of a polypeptide according to claim 1.

28. A binding protein that specifically binds to an epitope of a polypeptide according to claim 1.

29. An isolated polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 21-175 of SEQ ID NO:2.

30. An isolated polynucleotide according to claim 29, wherein said polypeptide is at least 90% identical in amino acid sequence to residues 21-175 of SEQ ID NO:2.

31. An isolated polynucleotide according to claim 29, wherein said polypeptide further comprises a cysteine residue corresponding to amino acid residue 81 of SEQ ID NO:2.

32. An isolated polynucleotide according to claim 29, wherein said polypeptide further comprises a copper binding site corresponding to amino acid residues 74-78 of SEQ ID NO:2.

33. An isolated polynucleotide according to claim 29, wherein said polypeptide comprises amino acid residues 26-175 of SEQ ID NO:2.

34. An isolated polynucleotide according to claim 29, wherein said polypeptide comprises amino acid residues 21-175 of SEQ ID NO:2.

35. An isolated polynucleotide according to claim 29, wherein said polypeptide comprises amino acid residues 1-175 of SEQ ID NO:2.

36. An isolated polynucleotide according to claim 29, wherein said polypeptide is approximately 1 kb in length.

37. An isolated polynucleotide according to claim 29, wherein said polynucleotide is selected from the group consisting of,

a) a sequence of nucleotides from nucleotide 138 to nucleotide 587 of SEQ ID NO:1;

b) a sequence of nucleotides from nucleotide 123 to nucleotide 587 of SEQ ID NO:2;

c) a sequence of nucleotides from nucleotide 63 to nucleotide 587 of SEQ ID NO:2;

d) allelic variants of a), b), or c); and

e) nucleotide sequences complementary to a), b), c) or d).

38. An isolated polynucleotide according to claim 29, wherein said polynucleotide is from 742 to 881 nucleotides in length.

39. An isolated polynucleotide according to claim 29 comprising nucleotide 1 to nucleotide 525 of SEQ ID NO:14.

40. An isolated polynucleotide according to claim 29, wherein said polynucleotide is DNA.

41. An oligonucleotide probe or primer comprising 14 contiguous nucleotides of a polynucleotide of SEQ ID NO:14 or a sequence complementary to SEQ ID NO:14.

42. A method for detecting a genetic abnormality in a patient, comprising:

obtaining a genetic sample from a patient;

incubating the genetic sample with a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1, under conditions wherein said polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first reaction product;

comparing said first reaction product to a control reaction product, wherein a difference between said first reaction product and said control reaction product is indicative of a genetic abnormality in the patient.

43. A method for detecting zsig10 polypeptides comprising:

exposing a polypeptide containing sample to an antibody attached to a solid support, wherein said antibody binds to an epitope of a zsig10 polypeptide;

washing said immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig10 polypeptide, wherein the second antibody is associated with a detectable label; and

detecting the detectable label.

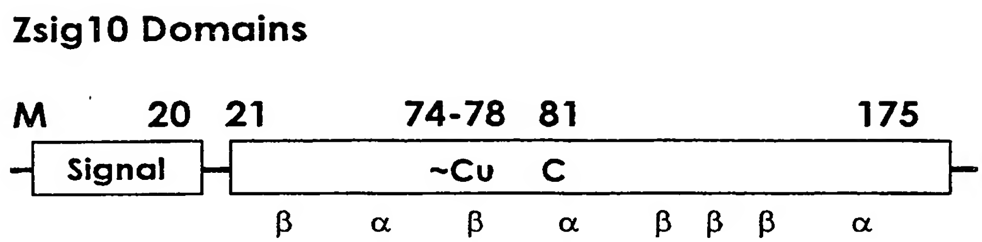

Fig. 1

2/2

```
         1         10         20         30         40
xlu76752 MQAGLSLVCL VLLCSALGEA VLKKPKKQAG TTDTKTDQ.. EPAPIKTKGL
         1         10         20         30         40         50
xlu82110 MQTGLSLACL VLLCSVLGEA ALRKPKRQAA ATDTNGAAKS EPAPVKTKGL
          1          10         20         30         40
Zsig10   ..MEKIPVSA FLLLVALSYT LARDTTVK.P GAKKDTKDS. .....RPKLP

         50         60         70         80         90
xlu76752 KTLDRGWGES IEWVQTYEEG LAKARENNKP LMVIHHLEDC PYSIALKKAF
                    60         70         80         90        100
xlu82110 KTLDRGWGED IEWAQTYEEG LAKARENNKP LMVIHHLEDC PYSIALKKAF
                   50         60         70         80         90
Zsig10   QTLSRGWGDQ LIWTQTYEEA LYKSKTSNKP LMIIHHLDEC PHSOALKKVF

        100        110        120        130        140
xlu76752 VADRMAQKLA QEDFIMLNLV HPVADENQSP DGHYVPRVIF IDPSLTVRSD
                   110        120        130        140        150
xlu82110 VADKMAQKLA QEDFIMLNLV HPVADENQSP DGHYVPKGIF IDPSLTVRSD
                  100        110        120        130        140
Zsig10   AENKEIQKLA .EQFVLLNLV YETTDKHLSP DGQYVPRIMF VDPSLTVRAD

        150        160        170        180
xlu76752 LKGRYGNKMY AYDADDIPEL ITNMKKAKSF LKTEL*
                   160        170
xlu82110 LKGRYGNKLY AYDADDIPEL ITT*...... ......
                   150        160        170
Zsig10   ITGRYSNRLY AYEPADTALL LDNMKKALKL LKTEL.
```

Fig. 2

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 98/05251

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/62 C07K14/47 C12N5/10 C07K16/18
G01N33/53 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NIELSEN H ET AL: "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites." PROTEIN ENG, JAN 1997, 10 (1) P1-6, ENGLAND, XP002072638 see the whole document	1-41,43
Y	TASHIRO K ET AL: "SIGNAL SEQUENCE TRAP: A CLONING STRATEGY FOR SECRETED PROTEINS AND TYPE I MEMBRANE PROTEINS" SCIENCE, vol. 261, 30 July 1993, pages 600-603, XP000673204 see abstract	1-41,43
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 1998	12/08/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

4

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	EMBL database Accession number U76752 Sive H., Bradley L. 05-DEC-1996 (Rel. 50, Created) "Progressive determination..." XP002072381 cited in the application see the whole document ---	1-41,43
A	SIVE H ET AL: "A sticky problem: the Xenopus cement gland as a paradigm for anteroposterior patterning." DEV DYN, MAR 1996, 205 (3) P265-80, UNITED STATES, XP002072379 cited in the application see abstract ---	1-41,43
A	MILLENNIUM BIOTHERAPEUTICS: "Progressive determination during formation of the anteroposterior axis in Xenopus laevis." CELL, JUL 14 1989, 58 (1) P171-80, UNITED STATES, XP002072380 cited in the application see abstract ---	1-41,43
A	EMBL database Accession number U82110 Aberger F. et al. 22-FEB-1997 (Rel. 51, Created) "A Xenopus cement ..." XP002072382 cited in the application see the whole document ---	1-41,43
A	YOKOYAMA-KOBAYASHI M ET AL: "A SIGNAL SEQUENCE DETECTION SYSTEM USING SECRETED PROTEASE ACTIVITYAS AN INDICATOR" GENE, vol. 163, 1995, pages 193-196, XP002053953 see the whole document ---	1-41,43
E	WO 98 11217 A (KATO SEISHI ;PROTEGENE INC (JP); SEKINE SHINGO (JP); SAGAMI CHEM R) 19 March 1998 see abstract; claims 1-3; figures SEQ.9,18,27 -----	1-8, 12-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/US 98/05251

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9811217 A	19-03-1998	AU 4220797 A	02-04-1998